Structural dynamics of PAH molecules upon energetic photon or ion interactions

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Chapter 7

Summary

PAHs are highly stable molecules which consist of a skeleton of six-membered rings of carbon atoms and have hydrogen atoms at the edges. The existence of PAHs in the interstellar medium (ISM) has important consequences for astrochemistry and astrophysics. The presence of PAHs influences many processes in space, especially in those regions where stars and planets form. Almost everywhere in space, the infrared spectra of PAHs are a diagnostics tool to probe the local physical conditions.

In this thesis, a series of pioneering experiments was performed on several PAHs, providing a tool set of methods which (a) simulate space conditions in the lab and (b) calibrate the probe which PAHs form by themselves. In the introduction a few key questions were formulated. In this summarizing chapter the main answers to these questions are presented.

How does the fragmentation of a PAH molecule depend on the amount of excitation energy deposited by ions?

To address this question an elegant experimental method was utilized which directly links the fragmentation of a molecular target to the energy deposition by protons. In chapter 2 anthracene ($\text{C}_{14}\text{H}_{10}$) ionization and fragmentation after double-electron transfer to 5 keV protons was studied. The excitation energies leading to the most relevant dissociation and fission channels of the resulting molecular dication were measured directly. To interpret the experimental data, Density Functional Theory (DFT) calculations on the structure of the molecular potential energy surface were performed.

This combined experimental and theoretical approach led to the following main conclusions: (i) The fragmentation of $\text{C}_{14}\text{H}_{12}^{2+}$ is dominated by emission of $\text{C}_2\text{H}_2$, which requires an excitation energy of 10.2 eV. By means of DFT calculations this channel is linked to a low energy pathway via isomerization to benz[a]azulene, which is associated with a barrier of 4.6 eV. (ii) Emissions of H and 2H/H$_2$ are associated with excitation energies between 4 and 14 eV. The calculated pathway
for molecular H\textsubscript{2} emission is associated with a barrier of 5.0 eV, while sequential loss of two H atoms requires substantially higher amounts of energy. (iii) Emission of C\textsubscript{3}H\textsubscript{x}+ is dominating over emission of C\textsubscript{2}H\textsubscript{x}+, with excitation energies of 12.9 and 13.6 eV, respectively. This is also consistent with the structure of the potential energy surface.

How much energy is actually set free when a PAH dissociates?

In order to answer this specific question a new experimental setup was built, which allows for the study of the dissociation dynamics of molecules upon interactions with energetic ions. In this new experimental approach a seeded jet was crossed with a focused ion beam in the center of a recoil ion momentum spectrometer. The fragment kinetic energies can be determined considerably more accurate than for the case of mere coincidence time-of-flight spectrometers. In chapter 3, 30 keV He\textsuperscript{2+} collisions with naphthalene (C\textsubscript{10}H\textsubscript{8}) molecules were studied. The following conclusions were drawn: (i) In fission reactions involving two cationic fragments, typically kinetic energy releases of 2-3 eV are observed. Fragmentation upon collisions with keV ions is much more violent than the corresponding photofragmentation with energetic photons. (ii) The diversity of naphthalene fragments in terms of composition, charge state, and kinetic energy proves that ion-induced PAH dissociation provides a feedstock of energetic hydrocarbonic species in the cold ISM. (iii) This feedstock can influence several molecule formation processes in the cold ISM and facilitates growth of small hydrocarbon species on pre-existing PAHs.

Can atomic hydrogen attachment to PAHs act as a buffer, protecting PAH molecules against fragmentation upon strong resonant excitation?

In chapter 4, we have investigated the response of superhydrogenated gas-phase coronene cations upon soft X-ray absorption. Carbon 1s-\pi^* transitions were resonantly excited at \(h\nu=285\) eV. The resulting core hole is subsequently filled in an Auger decay process, with the excess energy being released in the form of an Auger electron. In this way, highly excited dications are formed. These dications cool down by hydrogen emission in competition with infrared emission.

Dissociation and transition state energies for several H-loss channels were computed by means of DFT. Using these energies as input into an Arrhenius-type cascade model, very good agreement with the experimental data is found. The results have important implications for the survival of polyaromatic hydrocarbons in the interstellar medium and reflect key aspects of graphene hydrogenation: (i) Superhydrogenation of PAHs implies removal of bonding \pi electrons and leads to local puckering of the graphene-like planar honeycomb structure. Not only are the binding energies of the additional hydrogen atoms relatively low (\(\approx 2.5\) eV), but as a consequence of superhydrogenation, also the C skeleton is locally weakened. Despite this weakening, superhydrogenation clearly acts as a protection mechanism to PAH molecules. (ii) Superhydrogenation may explain the existence of large
amounts of PAHs in the interstellar medium, as de-excitation by H loss protects the C skeleton from breaking apart.

**How do PAHs respond to inner shell photoexcitation or ionization?**

In chapter 5 the response of pristine gas-phase coronene cations $C_{24}H_{12}^+$ upon soft X-ray photoabsorption is investigated for scanning the photon energy around the carbon K-edge. Inner shell photoabsorption followed by an Auger decay leads to the formation of intermediate $C_{24}H_{12}^{2+}$ and $C_{24}H_{12}^{3+}$ cations. The Auger mechanism and (below threshold) the excitation into unoccupied molecular orbitals leaves these intermediates internally hot. The high excitation energies lead to swift deexcitation, mainly by means of hydrogen loss.

We developed a statistical cascade model to simulate the experimentally obtained dehydrogenation states. The main ingredient for such a model are dissociation energies for subsequent H loss steps. These energies were calculated by means of DFT. The model is however limited to neutral H or H$_2$ loss from coronene dications and does not explicitly include charge separation processes, predicted to occur for higher charge states.

An analysis of the average dehydrogenation revealed that the intermediate $C_{24}H_{12}^{3+}$ trication is subject to a competition of neutral H loss and asymmetric fission leading to H$^+$ loss, with the neutral channel dominating and the fission channel being strongest for internally hot parent ions.

Many of the experimental data sets were interpreted with the help of DFT calculations. A direct comparison between DFT calculations and experiments relies on the assumption that all of the electronic excitation energy redistributes efficiently to vibrational degrees of freedom. As this coupling occurs on femtosecond timescales, femtosecond pump-probe experiments on PAHs are called for. As a first step, such a pump-probe experiment was succesfully performed on a pentapeptide in chapter 6.

In this experiment, our tandem mass spectrometer was combined with a 780 nm fs-laser system to study photoionization and photofragmentation of trapped protonated leucine enkephalin cations for laser intensities between $2 \times 10^{13}$ Wcm$^{-2}$ and $1 \times 10^{14}$ Wcm$^{-2}$ and pulse durations of 15 fs. In this intensity range, the transition from multiphoton ionization and excitation to tunneling ionization is expected to occur. The observed partial ion yield curves as a function of laser intensity exhibit a power-law dependence, indicating multiphoton absorption to be the dominant mechanism. The delay time dependent partial ion yields of almost all fragmentation channels show a broad but distinct maximum at a delay-time of approximately 750 fs. The particularly flat appearance of the pump-probe curves suggests that not a single resonance, but a broad distribution of resonances is involved.
Nederlandse samenvatting

Al sinds het begin van de mensheid zijn we nieuwsgierig naar de werking van het heelal. De prachtige sterrenhemel, het onveranderlijke dag-nachtritme en de verschillende vormen van de maan verwonderen ons al millennia lang. Al duizenden jaren geleden begonnen we de bewegingen van hemellichamen bij te houden om daarmee hun bewegingen in de toekomst te voorspellen. Zo kon men toen al zons- en maansverduisteringen voorspellen. In de zeventiende eeuw werd de eerste telescoop gebouwd. Tegenwoordig hebben we grote telescopen op aarde en aan boord van satellieten (figuur 7.1) waarmee we het heelal steeds nauwkeuriger kunnen observeren, maar elke nieuwe observatie levert naast antwoorden ook steeds nieuwe vragen op.

Als we met het menselijk oog kijken, zien we maar een heel klein deel van al het licht dat uitgezonden wordt door het heelal. Röntgen, ultraviolet (UV) en infrarood (IR) zijn soorten licht die onwaarneembaar zijn voor het menselijke oog. Om dat licht te zien, zijn speciale telescopen ontwikkeld, zoals bijvoorbeeld Spitzer (zie figuur 7.1). Naar welk deel van het heelal we deze telescoop ook richten, we zien overal infrarood licht. Net als het zichtbare licht bevat ook het infrarode licht verschillende kleuren. Maar waar komt al dat licht vandaan?

De verklaring hiervoor is dat er overal in de ruimte moleculen moeten zijn. Als je zou kunnen inzoomen op een molecuul, dan zou je zien dat het molecuul continu aan het trillen is. Een dergelijke trilling kan worden gedempt door de energie van de trilling om te zetten in de energie van een lichtdeeltje, een foton. Kleine verschillen tussen moleculen leiden tot kleine verschillen in de 'kleur' van het licht dat ze uitzenden. Zo vormt het infrarood de vingerafdruk van het molecuul!

Het licht dat we zien met behulp van telescopen toont de vingerafdrukken van een speciale familie van moleculen: polycyclische aromatische koolwaterstoffen (PAHs) (zie figuur 7.2). Dit zijn moleculen die bestaan uit waterstof- en koolstofatomen. De koolstofatomen zijn geordend in ringen en vormen het geraamte van de PAHs. De waterstofatomen zitten aan de randen. Zo krijgen de moleculen de vorm van een honingraat.

De ontdekking van deze moleculen is om twee redenen van zeer groot belang. In het heelal heeft de aanwezigheid van deze moleculen gevolgen voor de omgeving waarin ze zich bevinden. De precieze reikwijdte hiervan is het onderwerp van dit onderzoek. Wat gebeurt er bijvoorbeeld als een PAH uit elkaar valt? Kan een PAH überhaupt lang overleven in het heelal? En hoe beïnvloedt de aanwezigheid
van PAHs de vorming van andere moleculen, met name waterstof? Welke gevolgen heeft dit uiteindelijk voor de vorming van sterren?

De tweede reden is dat het licht dat PAHs uitzenden ons heel veel kan vertellen over hoe een bepaalde omgeving in het heelal eruit ziet. Als de omgeving van de PAH verandert, leidt dat namelijk weer tot kleine veranderingen in het licht dat wij waarnemen. De PAHs vormen dus eigenlijk kleine meetinstrumentjes, waarmee we de omstandigheden in het heelal, het ruimteweer, kunnen bepalen.

Om een goed beeld te krijgen van de rol die PAHs spelen in het heelal en om te weten te komen wat de informatie die de PAHs ons toezenden precies betekent, is het belangrijk dat er laboratoriumexperimenten worden gedaan. In die experimenten wordt steeds een stukje ruimte nagebootst en vervolgens wordt er nauwkeurig gekeken wat er gebeurt. In dit proefschrift wordt een aantal vragen beantwoord met behulp van dat soort experimenten.

**Hoeveel energie is er nodig om een PAH kapot te laten gaan?**

Om dit vast te stellen hebben we een experiment uitgevoerd waarbij een proton botst met antraceen (een PAH die bestaat uit drie ringen). We zorgen ervoor dat we de snelheid van het proton voor de botsing heel nauwkeurig weten. Na de botsing meten we de snelheid weer. Het verschil tussen de snelheid voor en na de botsing vertelt ons hoeveel energie er is overgedragen aan het antraceen. Tegelijkertijd meten we ook heel nauwkeurig wat er gebeurt met het antraceen. Blijft het heel?
Nederlandse samenvatting

Of valt het uit elkaar? En zo ja, hoe dan precies? Met deze methode leggen we dus een direct verband tussen de hoeveelheid energie die we in het molecuul stoppen en hoe het uit elkaar valt.

We hebben gezien dat het molecuul op drie manieren uit elkaar kan vallen, afhankelijk van de hoeveelheid energie die in het molecuul is gestopt. De eerste manier is het afdampen van een klein deel van het anthracene, bijvoorbeeld een enkel waterstofatom of een klein molecuul. De tweede manier is het splijten van het molecuul in twee delen en de derde is een combinatie van afdampen en splijting. Manier één vergt de minste energie, manier drie de meeste.

**Hoeveel energie komt er vrij als een PAH uit elkaar valt?**

Deze vraag wordt beantwoord met behulp van een experiment dat speciaal daarvoor is opgebouwd. Hier botsen we He\(^{2+}\)-deeltjes met naftaleen (twee ringen). We zorgen ervoor dat we heel erg nauwkeurig weten waar deze botsing plaatsvindt. Als door de botsing het naftaleen uit elkaar valt in bijvoorbeeld twee elektrisch geladen delen, dan zullen beide delen een zekere snelheid hebben gekregen. De massa van deze deeltjes wordt gemeten door ze heel hard naar een detector toe te trekken. De tijd tussen de botsing en de aankomst bij de detector vertelt ons hoe zwaar de deeltjes zijn. De plaats waar de deeltjes de detector raken is een maat voor de energie die ze mee hebben gekregen toen het naftaleen in stukken brak.

We hebben gemeten dat de PAH-fragmenten een zodanig hoge energie hebben
dat dit grote gevolgen kan hebben voor de ruimte waarin ze zich bevinden. De energieën zijn namelijk hoog genoeg om nieuwe moleculen te vormen en misschien zelfs nieuwe PAHs.

**Hoe verandert de stabilitéit van een PAH als ze omringd worden met extra waterstof?**

In de ruimte is waterstof het meest voorkomende atoom. PAHs zullen dan ook vaak waterstofatomen tegenkomen. Is het mogelijk dat er waterstof aan de PAHs blijft plakken?

In hoofdstuk 4 en 5 wordt een experiment (zie figuur 7.3) beschreven waarin deze vraag centraal staat. Als PAH gebruiken we coroneen (7 ringen, zie figuur 7.2). De coroneenmoleculen worden eerst gevangen met behulp van elektrische velden. Het wolkje coroneenmoleculen dat dan ontstaat wordt besproeid met waterstofatomen. Na een tijdje wordt gekeken hoe de inhoud van het wolkje veranderd is. Het blijkt dat het mogelijk is de PAH te 'superhydrogeneren', dat wil zeggen extra waterstofatomen aan de PAH te plakken.

Wanneer een dergelijk proces in de ruimte plaatsvindt, hoe reageert het PAH molecule dan op de omstandigheden die in de ruimte voorkomen? Wij hebben
PAHs beplakt met waterstof en dan bestraald met een speciaal soort licht dat het beste te vergelijken is met het licht dat bij een röntgenfoto wordt gebruikt. Dat licht zorgt ervoor dat we in één keer heel veel energie in het molecuul kunnen stoppen. Na deze bestraling kijken we weer heel nauwkeurig naar de inhoud van het wolkje.

Deze experimenten hebben geleid tot opzienbarend conclusies. Het blijkt dat waterstof een soort beschermende laag vormt voor de PAHs: het molecuul zelf valt minder gemakkelijk uit elkaar als gevolg van het aanwezige waterstof. Dit kan verklaren dat PAHs ook overleven in het gure ruimteweer.

Toekomstige experimenten aan PAHs

Alle processen die hierboven zijn beschreven gebeuren heel erg snel, meestal royaal binnen een picoseconde (een miljoenste van een miljoenste) seconde. Als we precies willen weten hoe een dergelijk proces in zijn werk gaat, moeten we dit eigenlijk filmen. Het verschil met een normale film is immens. Moleculen zijn namelijk erg klein en processen gaan extreem snel. Je hebt dus een camera nodig die per milliseconde een miljoenmaal een miljoen beelden kan maken. Tevens moet de resolutie zo goed zijn dat je een miljoenste van een millimeter kan zien.

Om toch zo’n film te kunnen schieten, gebruiken we licht, afkomstig van hypermoderne lichtbronnen in Heidelberg, Hamburg en Berlijn. Dit licht moet fel zijn, de kleur (golflengte) moet heel nauwkeurig te kiezen zijn, en de lichtflitsjes moeten erg kort zijn. In hoofdstuk 6 van dit proefschrift wordt een prototype experiment beschreven waarin we een snel moleculair proces in een molecuul vastleggen.

Het bestuderen van ultrasnelle processen in PAHs met behulp van deze moderne technieken is een grote uitdaging voor de toekomst.
Dankwoord

De volgende mensen ben ik dankbaar voor hun rol in de totstandkoming van dit proefschrift of voor elke andere rol die zij de afgelopen vier jaar hebben gespeeld.

- Ronnie Hoekstra en Thomas Schlathölter voor de dagelijks begeleiding van mijn onderzoek. Jullie waren altijd beschikbaar voor overleg over de experimenten in het lab en de interpretatie van de data. Ook hebben jullie veel tijd gestoken in het lezen en verbeteren van mijn manuscripten. Dank daarvoor!


- Stephanie Cazaux voor de prettige samenwerking in het kader van het superhydrogenatieproject.

- Ook de mensen van de werkplaats en de tekenkamer ben ik veel dank verschuldigd. Ik ben erg onder de indruk van de snelheid waarmee mijn vage schetsen werden omgezet in professionele werktekeningen en vervolgens in een mooi eindproduct. Ook kon ik met kleine klusjes, vaak op ongunstige tijdstippen, altijd terecht.

- Serge Martin, Richard Brédy, Li Chen en Jérôme Bernard van de universiteit van Lyon voor alle hulp bij de experimenten die ik in hun groep mocht uitvoeren.

- Robert Moshammer, Bettina Fischer en Nicolas Camus van het MPI in Heidelberg voor alle hulp bij de fs-laserexperimenten.

- Henning Zettergren van de universiteit van Stockholm voor alle hulp bij DFT-berekeningen.

- De leescommissie, Xander Tielens, Henrik Cederquist en Petra Rudolf, voor de nuttige feedback op mijn proefschrift.

- Mijn paranimfen: Berno Reitsma en Leon Boschman.
Verder zijn er veel mensen die wellicht niet direct hebben bijgedragen aan de totstandkoming van mijn proefschrift, maar die mijn tijd in Groningen wel een stuk leuker hebben gemaakt. De vrienden van de ‘weekendgroep’ en ‘Het gezag’ ben ik daar erg dankbaar voor.

Mijn teamgenoten van Donitas ben ik erg dankbaar dat ik niet ‘dankzij’, maar ‘ondanks’ hen mijn proefschrift heb kunnen afronden.

Tenslotte mijn ouders, broertje en zusjes voor alle steun voor en interesse in wat ik doe.
Co-authored publications

During my PhD research I contributed significantly to a number of publications of which I am co-author. In this appendix all co-authored publications are listed and in table 7.1 my contribution is elucidated:


<table>
<thead>
<tr>
<th>Paper</th>
<th>Preparations</th>
<th>Experiments</th>
<th>Analysis</th>
<th>Discussions</th>
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Table 7.1: Co-authored papers and contributions


Cross sections for energetic heavy-ion impact on protonated water clusters

Alexander von Zastrow • Rico Otto • Sébastien Jézouin • Jonathan Brox • Martin Stei • Olmo González-Magaña • Geert Reitsma • Thomas Schlathölter • Ronnie Hoekstra • Thorsten Best • Roland Wester

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Abstract Energetic impact of multiple ionized oxygen on protonated water clusters in the range of eight to twenty-one water molecules is investigated on the ZERNIKE–LEIF facility. The target water clusters are stored in a Paul trap and thermalized by cold buffer gas. This well-controlled approach allows for a direct measurement of the total inelastic cross section leading to trap-loss processes of the target ions.

1 Introduction

Energetic ion impact in water is of central importance for an understanding of radiation damage of biological tissue on a molecular level (see [1] and references therein) and radiation treatment of cancer with heavy ions [2]. It has been pointed out that free charge carriers and radicals, set free when ionizing radiation passes through the aqueous tissue environments might chemically attack biomolecules. These secondary reactive species are known to cause comparable harm to direct ionization or fragmentation of the biomolecules (e.g., [3, 4]). In particular, it has been demonstrated that resonances in the fragmentation cross section of biomolecules by slow electrons go along with site-specific bond-breaking [5]. The primary processes that lead to the formation of such attackers still await elucidation. In particular, the balance between direct and indirect radiation damage is influenced by the nature of the ionizing radiation and the presence of radical-scavenging substances [6]. As a starting point, a quantitative and microscopic understanding of what happens when an energetic heavy ion impacts on water is therefore highly desirable. In this work, we approach this question by investigating energetic ion impact on individual protonated water clusters of size n = 8…21. Storage of these target clusters in an ion trap allows us to measure the absolute total destruction cross section for impact at various well-defined energies.

2 Experimental setup

The experiments described here have been carried out at the Pauliże experimental station of the ZERNIKE–LEIF facility. The main components of the experimental
apparatus have been described earlier [6, 11]. A simplified scheme of the apparatus is shown in Fig. 1.

2.1 Ion production and storage

Water cluster ions are continuously produced in a needle corona discharge in humidified air similar to reference [7]. They are subsequently inserted in vacuum through a biased capillary and an ion funnel. Following a differential pumping section, a quadrupole mass selector allows to pick various ranges of cluster sizes. Selection and trapping of a single-cluster size only could not be achieved, which is most probably due to unimolecular or collisional dissociation of the clusters in or after the mass selector. A gate electrode allows operating the resulting beam of target ions in a pulsed mode for loading of the ion trap. The trap is a commercially available Paul-type quadrupole [10] which features two holes in the endcap electrodes used for loading and unloading the trap with the target ions, and two holes on opposite sides of the ring electrodes, which allow to pass the highly energetic projectile ion beam. Parent clusters in the range \( n = 8–21 \) (\( n \) being the number of water molecules in the cluster) can be produced. The abundance of magic number clusters at \( n = 21 \) is clearly enhanced, as observed in previous studies [8, 9], but a significant enhancement is also observed at \( n = 15 \), which was not observed as clearly earlier. We will refer to both clusters as magic clusters in the following, as they both show distinct behavior from other cluster sizes.

2.2 Energetic multiply charged projectile ion beam

The projectile ions are produced in an electron cyclotron resonance type source on a platform potential of up to 15 kV. A selector magnet allows to pick ions in specific charge states. In order to make the most direct connection to applications of ion beams in cancer therapy, carbon ions would seem preferable [2]. However, for ease of producing intense stable ion beams, the experiment was carried out with fivefold-charged oxygen ions instead. We expect the difference to be of minor importance, as long-range interactions, which do not depend on the exact nature of the projectile, seem to dominate. A switchable deflector in the beam path allows to effectively switch the beam on and off for online background monitoring. We continuously monitor the beam current after passage through the Paul trap using a Faraday cup, as well as on the shield of an \( A = \frac{1}{2} \text{mm}^2 \) aperture in front of the trap. Thereby, we can deduce that the beam impinging on the aperture is significantly wider than the latter. Thus, the beam passing through the trap is essentially homogeneous, with a diameter defined by the aperture. Typical beam currents in the Paul trap are in the tens of Nanoampere regime, allowing for current monitoring even in pulsed mode.

2.3 Buffer gas cooling

The stored cluster ions in the trap can be cooled to liquid nitrogen temperatures by collisions with a helium buffer gas. Using a fast current monitor and switching on and off the energetic ion beam, we find that a significant fraction of the beam current is absorbed by the buffer gas if the latter is present in the trap while the beam is on. Therefore, buffer gas cooling was limited to the time before the clusters are exposed to the ion beam. While we have verified that the trap lifetime is significantly longer than all measurement timescales with and without buffer gas, the trapping efficiency of fragment ions might be adversely affected by the lack of buffer gas. Therefore, we expect only a fraction of the actual fragment ions to be trapped and detected in the experiment.

2.4 Detection of mass spectra and signal processing

After storage and exposure to the projectile ion beam, the clusters are extracted from the Paul trap by means of a pulsed extraction voltage and enter a linear time of flight (TOF) spectrometer, where they are finally detected on a multichannel plate. The TOF mass spectrum is recorded by a computer-based oscilloscope system. A typical TOF spectrum is shown in Fig. 2. Spectra are acquired in cycles of three, one spectrum with and two without exposure to the ion beam, which we denote by \( S_1 \) and \( S_0 \), respectively, and one exposure \( BG \) with no water clusters loaded into the Paul trap. The latter acquisition accounts for any processes that the highly energetic projectiles cause in the buffer gas, background gas, or upon hitting the trap electrodes. The ratio

\[
\frac{S_1 - BG}{S_0}
\]

then characterizes the effect of the energetic ion beam on the trapped clusters, a value of \( \hat{S} > 1 \) or \( \hat{S} < 1 \) indicating...
growth and loss of a species, respectively. In particular, this quantity is insensitive to temporal drifts in the cluster production efficiency, which may be caused, e.g., by variations of relative humidity in ambient air. The quantity \( \tilde{S} \) is calculated TOF-bin-wise for every triple of acquisitions, averaged over typically 300 iterations for every set of measurements and stored for later analysis. After carrying out a TOF-to-mass calibration, we obtain the fraction \( \frac{N}{N_0} \) of remaining ions of a specific cluster size by integration of \( \tilde{S} \) over a suitable TOF window.

### 3 Measurement of absolute cross sections

Absolute cross-section measurements based on decay-rate measurements in a trapped sample have been demonstrated earlier for such diverse processes as photodetachment with continuous [12] or pulsed lasers beams [13] as well as electron-impact ionization [14]. In the present work, we build on this measurement scheme, with the slight complication that here significant beam flux variations over the course of the experiment have to be taken into account. When a sample of \( N_0 \) ions is exposed to a projectile current density \( j \) over a time \( \tau \), we define the exposure as

\[
\Phi = \frac{j \cdot \tau}{q},
\]

where \( q \) is the projectile charge. The loss of target ions from the trap can be written as

\[
N(\Phi) = N_0 e^{-\sigma \Phi},
\]

where \( \sigma \) is the total cross section for processes leading to trap loss of the initial target ion. The current density is related to the total current \( I_{FC} \) measured on the Faraday cup via

\[
j = \frac{I_{FC}}{A},
\]

where \( A \) is the area of the aperture which defines the projectile beam shape. If we therefore measure the fraction \( \frac{N}{N_0} \) of remaining clusters depending on the exposure \( \Phi \), we can derive the cross section from a least squares’ fit according to

\[
\log \frac{N}{N_0} = -\sigma \Phi,
\]

as shown exemplarily in Fig. 3. The density distribution of the ions in an unbiased Paul trap (i.e., with no DC potential) is well known in the case of negligible space charge [15]. It is interesting to note, however, that as long as the corresponding width is small compared with the energetic ion beam, which is well fulfilled in our experiment, the actual value of the cloud width is not directly relevant to the derivation of the cross section.

### 4 Results and discussion

#### 4.1 Absolute fragmentation cross sections

Measurements similar to the ones shown in Fig. 3 have been carried out for projectile energies of 7.5, 10.0, 12.5, and 15.0 keV/q. The results are summarized in Table 1.

#### 4.2 Scaling with cluster size

The dependence of the observed cross section on the cluster size is shown in Fig. 4. Empirically, we find a linear scaling for nonmagic clusters at all energies. The cross sections for magic parent clusters, however, are significantly smaller than the general trend implied by this scaling would suggest.
4.3 Energy dependence

For any given target mass, we can also extract the scaling of the cross section with increasing projectile energy \( E_0 \), see Fig. 5. We can fit an empirical power law according to

\[
\frac{r}{E^{d_0}} = \text{constant}
\]

We thereby obtain the exponents shown in Table 2, which except for the case of \( n = 20 \), are very well consistent with a \( 1/E^2 \) behavior for both magic and nonmagic clusters, the error-weighted average being \( \delta = 1.95 \) or \( \delta = 2.06 \), excluding or including the \( n = 20 \) value.

4.4 Product branching

Although charge transfer processes can sometimes be observed in the mass spectra, loss of individual neutral water molecules seems to be by far the dominant output channel. Fragment ions lacking one or several water molecules are observed, as well as a small amount of charge transfer products. A strong contribution of other mechanisms, the products of which (e.g., very light clusters) could be undetectable in the present scheme, can be ruled out based on the observation that the integral value of \( \tilde{S} \) over the full spectrum, is not reduced strongly with increasing exposure. However, the exact collection efficiency of fragments in the trap is largely unknown, and further fragmentation processes of trapped fragments are relatively likely. We therefore make no attempt to quantitatively analyze these branchings. Qualitatively, the most apparent daughter clusters are those that are close in size to the parent, indicating that the fragmentation proceeds dominantly via loss of individual water molecules.

5 Interpretation

A linear scaling with cluster size is to be expected if the scattering can be considered to happen primarily on individual water molecules, with the remaining constituents of

<table>
<thead>
<tr>
<th>Cluster size ( n )</th>
<th>Power-law exponent ( \delta )</th>
<th>( \Delta\delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2.06</td>
<td>0.18</td>
</tr>
<tr>
<td>11</td>
<td>1.95</td>
<td>0.12</td>
</tr>
<tr>
<td>14</td>
<td>2.06</td>
<td>0.10</td>
</tr>
<tr>
<td>15</td>
<td>1.89</td>
<td>0.11</td>
</tr>
<tr>
<td>20</td>
<td>2.43</td>
<td>0.09</td>
</tr>
<tr>
<td>21</td>
<td>1.87</td>
<td>0.09</td>
</tr>
</tbody>
</table>

The given values for \( \Delta\delta \) denote the statistical fitting error.
the cluster acting merely as spectators, and the number of such potential scattering partners increasing linearly with cluster size. Alternatively, an additive quantity must be involved in the scattering. The change in binding energy associated with cluster growth seems largely negligible compared with the available energy transfer for hard-sphere-type collisions. On the other hand, multiple scattering within one cluster can be considered negligible. The observed energy dependence is atypical for both electronic and nuclear stopping in the energy range of interest, as well as for charge transfer processes. The latter also seem to play only a minor role based on the mass spectra after exposure. Taking together these observations and the fact that the observed cross sections are significantly larger than suggested by the geometrical target area associated with the water clusters [16], it seems reasonable to assume that the dominant processes leading to collision are based on long-range polarization-type interactions with the electric field of the fast-moving ion. The importance of such processes has already been highlighted for the case of swift projectiles [17].

6 Conclusion

In the experiments described in the present paper, we have obtained absolute cross sections for fragmentation processes of cationic water clusters under energetic ion bombardment. The dependence on cluster size and projectile energy has been determined in terms of power-law scalings. These scalings, the relatively large cross sections and the observed fragments lead us to a tentative interpretation in terms of long-range polarization-type interactions. As a result, mainly individual water molecules are expelled from the cluster. An influence of the surrounding cluster can be observed in terms of a larger stability with respect to fragmentation of magic number parent clusters. The precise mechanism behind this stability enhancement remains unclear and may be worth further studies for elucidation. Both the linear size scaling and the above interpretation suggest that the overall positive charge of the cluster is not crucial for the fragmentation process, and thus, similar results are to be expected for neutral or anionic clusters. It may therefore be of interest to investigate even larger clusters in order to make the connection to bulk water behavior, where precision models of ion stopping over a large range of energies are available [18]. Finally, we would like to note that the method employed in this work can readily be applied to the study of absolute cross sections for other fragmentation processes, e.g., in the context of direct fragmentation of biomolecules.

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Electron capture and deprotonation processes observed in collisions between Xe$^{8+}$ and multiply protonated cytochrome-C

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Electron-transfer processes in interaction between highly charged ions and multiply protonated proteins have been studied. Collisions between Xe$^{8+}$ at 96 keV and protonated cytochrome-C at selected charge state ($q$ from 15+ to 19+) result in mass spectra composed mainly of intact molecular ions. From the spectra, single and double electron capture processes by Xe$^{8+}$ from the protonated molecular ions were identified and the relative cross sections were measured. An unexpected process, the deprotonation process, was also observed. It is tentatively attributed to the loss of a proton induced by the strong electric field carried by the projectile ion in long-distance collisions. Upon charge variation of the molecular target from 15 to 19, the single and double electron capture cross sections remain nearly constant, while the relative cross section of the deprotonation process increases dramatically from 0.8% ($\pm 0.1\%$) to 17% ($\pm 1\%$). This strong charge dependency is explained by the decrease of the proton affinities with the charge. This proton removal process has not been observed previously. It seems to be specific to the long-distance Coulomb interactions between protons bound along the protein chain and the highly charged atomic ions.

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I. INTRODUCTION

For many decades, collision dynamics between highly charged ions (HCIs) and atomic or molecular targets in the gas phase have been investigated [1]. Most works on ion-neutral target collisions were performed in crossed beam configurations while a number of more recent studies have employed merged beam techniques [2,3]. Ion-molecular target collisions have been investigated extensively during recent years in order to gain structural information of (eventually multiply) charged molecules and to study their stability and fragmentation dynamics. In these experiments, the electron transfer from the target molecule to the HCI is the main interaction mechanism leading to ionization, excitation, and the subsequent dissociation of the molecules. Most of the molecules investigated so far could be easily brought into the gas phase by evaporation using a simple oven [4–8]. An entire class of molecules of biological interest, including DNA bases, sugars, small amino acids, porphyrins, etc. [9–13], has been studied in collisions with HCIs. To bring larger and generally more fragile biomolecules into the gas phase, more sophisticated molecular source techniques such as electrospray ionization (ESI) have to be employed. Recently, an experimental setup combining an ESI, a radiofrequency (RF) ion trap device, and a HCI source has been built at the Zernike-LEIF facility at the Kernfysisch Versneller Instituut (KVI), University of Groningen. This experimental investigation of collisions between HCIs and stored small protonated peptides (Leucineenkephaline, bradykinin . . . ) [14] has revealed two main classes of interaction processes: (i) electron capture processes at long collision distance leading to little excitation of the target and even formation of intact peptide dications; (ii) collisions at closer distance leading to higher energy deposition and extensive fragmentation of the peptide. This pioneering work has opened the way for exploring a new research field on interactions between HCIs and larger biomolecules such as proteins.

Electron transfer in ion-ion collisions at much lower interaction energies (a few eV) has already been studied intensively using ion trap mass spectrometry. Experimental techniques such as electron-transfer dissociation (ETD) and negative ETD are now routinely used for analytical purposes [15–20]. Gas phase charge transfer processes involving protonated peptides or proteins are directly related to the proton or electron binding energies of such species [21,22]. To measure these binding energies, photoionization (e.g., using synchrotron radiation) and electron impact ionization techniques have been employed [21–24]. As expected, for a given protonated protein the ionization potential ($I_p$) and electron affinity (EA) were found to depend sensitively on the charge $q$, i.e., the number of protons bound to the protein. For example, for a series of small proteins with mass between 1000 and 3500 amu (insulin, melittin), the $I_p$ follows a simple linear law with $q$, $I_p$ (eV) = 9.8 + 1.1$q$ [25]. For larger proteins such as cytochrome-C (denoted as cyt-C hereafter) ($m=12 \times 10^3$ amu), the charge effect on the $I_p$ is weaker and the linear $q$-dependent term is approximately 0.2$q$ (eV) [23]. Besides the roughly linear variation feature of $I_p$ as a function of $q$, Giuliani et al. [26] observed a small plateau in the $I_p(q)$ curve for protonated cyt-C, corresponding to structural modifications of the protein as demonstrated by Clemmer and Jarrold using ion mobility spectrometry [27].

In this paper, we study collisions between HCI Xe$^{8+}$ at 96 keV and a multiply protonated biomolecule, the protein cyt-C, with charge state or the number of protons ranging from $q = 15+$ to 19+. The goal of this study is to address the electron transfer and relaxation mechanisms occurring in fast interactions involving two highly charged species. Single and double electron transfer from the highly charged...
protein to a point-charge projectile ion was observed. The total electron-transfer cross section was calculated with the classical over-the-barrier model under the assumption of a simple linear molecular conformation. An unexpected process, the deprotonation process, was also observed. The charge dependency of its measured cross section was found strongly correlated to the proton affinity (the minimum energy to remove a proton from the molecule) of the protonated cyt-C.

II. EXPERIMENT

The experimental setup (Fig. 1) has been described in detail elsewhere [28] and only a short description is presented here. A homemade ESI source was used to produce the protonated cyt-C molecules. A 30-μM methanol solution with typically 1% acetic acid contained bovine cyt-C molecules, denoted $M$, of mass 12 229 amu (Sigma-Aldrich). It was dispersed by electrospray into fine aerosols through a capillary heated to 100 °C. After solvent evaporation in vacuum, protonated molecular ions, denoted $[M + q\ H]^q+$ with $q$ ranging from 12+ to 20+, were formed with the attachment of $q$ protons on the basic residues of the cyt-C. The density of free protons available in the solution could be controlled by varying slightly the density of the acetic acid. The higher the acid amount, the higher the proton density and therefore the higher the charge state $q$ attained by the protonated molecules from the ESI source. The ions were guided by a first quadrupole and selected in charge state through a second quadrupole which served as a mass filter. The selected ions cyt-C $[M + q\ H]^q+$ were then accumulated during about 500 ms in a Paul trap where a He buffer gas was injected in order to trap and cool the ions (pressure up to $10^{-3}$ mbar). A time diagram of the sequence of the experiment is presented in Fig. 2. At the end of the accumulation phase, the incident molecular ion jet was blocked by applying a voltage of 25 V to the einzel lens located between the mass filter and the trap, and the He gas injection valve was closed. With the buffer gas off, the pressure in the trap dropped quickly to approximately $10^{-6}$ mbar in less than 0.5 s of storage time. An ion beam of Xe$^{8+}$ (10 nA) extracted from an electron cyclotron resonance (ECR) source and accelerated to 96 keV was sent into the trap to collide with the stored molecular ions. It was collimated and guided to the center of the trap through a pair of 2.4-mm apertures drilled in two opposite sides of the ring electrode. The number of protonated cyt-C ions stored in the trap was estimated to be on the order of $10^{5}$ ions. Good vacuum condition was therefore necessary to maintain low relative collision cross section (1%) between the Xe$^{8+}$ beam and the background gas composed of residual He gas and neutrals coming from the ESI source. A typical irradiation time of 1.4 s was necessary to deplete about 20% of the stored parent ion population via collision induced charge exchange or dissociation. After the irradiation phase, a pulse of He buffer gas was injected during 0.1 s in order to cool down the energetic dissociation products and quench further dissociation processes. After the whole sequence of accumulation-storage-irradiation-cooling, ±200-V bias voltages were applied to the endcaps of the Paul trap. The stored ions, including intact molecular ions and fragments, were extracted and sent into a time-of-flight (TOF) mass spectrometer of resolution $M/\Delta M \approx 200$. A postacceleration voltage of 5 kV was applied in front of the multichannel plate detector in order to improve the detection efficiency. The detector signal was recorded with a 1-GHz digitizer over 65 μs of TOF range. The typical duration to record a spectrum was about 1 h. In a raw TOF spectrum, peaks due to collisions of Xe$^{8+}$ with the background gas were observed mainly in the low mass-over-charge range below 100 amu. Their contribution to the mass spectra was corrected by the subtraction of a background spectrum obtained without protein accumulation in the trap (see Ref. [28] for details).

III. RESULTS AND DISCUSSION

A. Spectrum analyses

Figure 3 shows the mass spectra obtained with or without Xe$^{8+}$ irradiation in experiments using fast Xe$^{8+}$ ions (96 keV) and trapped protonated protein ions cyt-C $[M+18\ H]^{18+}$. Figure 4 shows the mass spectra obtained in collisions between Xe$^{8+}$ and cyt-C $[M+q\ H]^q+$ ($q = 15–19$). In the large mass-over-charge range (500–900 amu), the spectra exhibit only a few peaks showing similar features as the spectra obtained for multiply protonated proteins in ETD experiments [29]. For example, in Fig. 4(a) corresponding to collisions with $[M+15\ H]^{15+}$ ions, three main peaks are observed. The dominant peak is assigned to the parent ions $[M+15\ H]^{15+}$ and two other peaks at lower mass-over-charge ratio are assigned to

![FIG. 1. (Color online) Experimental setup.](image-url)
ELECTRON CAPTURE AND DEPROTONATION PROCESSES . . . PHYSICAL REVIEW A 89, 012707 (2014)

Molecular ions undergoing dissociation with the loss of a small neutral fragment could have contributed to the broadening of the observed main peaks. However, in the case of cyt-C, it is expected that dissociation occurs mainly along the backbone leading to the loss of a-b type or c-z type fragments \(^{18}\) with a typical mass large enough to allow the remaining charged fragment to be resolved from the parent ion peak. On the other hand, dissociation of the molecular ions into two or more charged fragments could lead to the production of fragments with mass-over-charge ratio in the domain of our mass spectrum. However, due to the large size of the molecular chain, hundreds of scission sites are possible leading to the broad unresolved background of the spectrum. Therefore, contribution of fragments with the same mass-over-charge ratio as the main peaks is mixed in the background. These arguments allow us to confirm the assignment of the peaks to intact molecules. In all spectra obtained with cyt-C \([M + qH]^+\) \((16 \leq q \leq 19); \text{Fig.} \, 4(b)–4(e))\], peaks assigned to intact parent ions \([M + qH]^+\) and intact up-charged ions \([M + qH]^{(q+1)+}\) and \([M + qH]^{(q+2)+}\) are observed. We attribute \([M + qH]^{(q+1)+}\) and \([M + qH]^{(q+2)+}\) to single electron capture (SEC) and double electron capture (DEC) processes by Xe\(^{8+}\) from the parent molecular ions. Three small fragment peaks can be identified and tentatively assigned. Among these peaks, one corresponds most probably to the monocharged heme molecular ion \((m = 617 \text{ amu})\) and two others to small monocharged fragments \((m = 535, 552 \text{ amu})\). To identify precisely all fragment peaks in the background, a mass spectrometer with high mass resolution up to 20 000 would be needed.

Similar as the example shown in Fig. 3(a), mass spectra of the other stored \([M + qH]^+\) \((q = 15–19)\) ions have been also measured without Xe\(^{8+}\) irradiation. From the spectra normalized to the same experimental conditions, i.e., the same number of trapped ions, the total counts \(N_{\text{SEC}}\) and \(N_{\text{DEC}}\) of the parent ions \([M + qH]^+\) measured with and without irradiation, have been obtained from the integral of the peaks (Table I). A population depletion ratio of the stored parent ions due to Xe\(^{8+}\) irradiation was calculated using \(\rho = (N_{\text{SEC}} - N_{\text{DEC}})/N_{\text{SEC}}\) and was found to be \(\rho = 17\%, 19\%, 20\%, 23\%,\) and \(24\% \,(\pm 1\%)\) for \(15 \leq q \leq 19\), respectively. This parameter is related tightly to the total interaction cross section in collisions between Xe\(^{8+}\) and \([M + qH]^+\). Typically, higher cross section should lead to larger depletion ratio. The dependency of the measured depletion ratio \(\rho\) on \(q\) suggests that the total cross section increases slightly with the charge of the protonated molecular ions. From Fig. 4, the counts \(N_{\text{SEC}}\) and \(N_{\text{DEC}}\) for the SEC and DEC processes leading to intact molecular ions were measured, respectively, from the integral of the peaks \([M + qH]^{(q+1)+}\) and \([M + qH]^{(q+2)+}\) (Table I). The relative cross section of these processes \(\sigma_{\text{SEC}}/\sigma_{\text{DEC}}\) was estimated from the ratio of \(N_{\text{SEC}}/N_{\text{DEC}}\) versus the total parent ion population depletion \((N_p - N_{\text{SEC}})/N_p\) and

\[
\begin{array}{cccccc}
 q & N_p & N_{\text{SEC}} & (N_p - N_{\text{SEC}})/N_p & N_{\text{SEC}} & N_{\text{DEC}} & N_p \\
 15 & 1216000 & 1010000 & 0.17 & 29000 & 10000 & 1600 \ 
 16 & 1216000 & 985000 & 0.19 & 23000 & 6500 & 11000 \ 
 17 & 1216000 & 973000 & 0.20 & 28000 & 11500 & 18000 \ 
 18 & 1216000 & 936000 & 0.23 & 30000 & 12500 & 36000 \ 
 19 & 1216000 & 924000 & 0.24 & 28000 & 10500 & 49000 \\
\end{array}
\]

TABLE I. Count (integrated area) of each peak after normalization of the spectra. \(N_p\): Parent ion peak \([M + qH]^+\) without irradiation; \(N_{\text{SEC}}\): Parent ion peak \([M + qH]^+\) with irradiation; \(N_{\text{SEC}}\) and \(N_{\text{DEC}}\): \(N_{\text{SEC}}\): peaks corresponding, respectively, to single electron capture \([M + qH]^{(q+1)+}\), double electron capture \([M + qH]^{(q+2)+}\), and deprotonation \([M + (q-1)H]^{(q-1)+}\) processes.
$\sigma'_{DEC} = N_{DEC}/(N_p - N_{pi})$. $\sigma'_{SEC}$ was found to vary from 14% to 9% (±1%) with increasing charge $q$ of the protonated molecules $[M + qH]^q+$. While $\sigma'_{DEC}$ varied slightly with $q$ from 5.0% to 4.0% (±0.5%).

Another intense peak can be observed in the mass spectra of Fig. 4. For instance, in Fig. 4(d) which depicts the results for collisions with $[M + 18H]^{18+}$, a peak is observed at the nominal mass over charge, 720 amu. This is close to the value expected for the intact molecular ions with a lowered charge, $q = 17$. Similarly, in spectra obtained with other trapped parent ions $[M + qH]^q+$, a peak at mass-over-charge ratio around that of the intact molecular ion with a charge $q = 1$ can also be noticed. The calibration of the mass spectra leads to an uncertainty in the mass determination of approximately two to three hydrogen atoms. Furthermore, due to the asymmetric shape of the peak, determination of the mass of the molecular ions with a precision up to the mass of hydrogen is impossible. Nevertheless, in the following, we tentatively attribute these peaks to the quasi-intact parent ions with the loss of one proton, $[M + (q - 1)H]^{q-1+}$. This process is labeled DP in Fig. 4 for “deprotonation”. This attribution will be discussed in more detail in a following section. From the measured counts $N_{DP}$ (Table I), we have estimated the relative cross section of this process, $\sigma_{DP} = N_{DP}/(N_p - N_{pi})$. It increases strongly with the charge of the target ions, from about 0.8% (±0.1%) for $q = 15$ to 17% (±1%) for $q = 19$.

The measured SEC, DEC, and DP processes leading to intact or quasi-intact molecules corresponding to about 20% (±2%) to 30% (±2%) ($\sigma'_{SEC} + \sigma'_{DEC} + \sigma'_{DP}$) of the delected cyt-C $[M + qH]^q+$ population with increasing charge $q$. Therefore, a major part of the delected molecular ions undergoes fragmentation after interaction with Xe$^{8+}$. A part of the fragmented population contributes to the broad background of the mass spectra and another part may have escaped from the trap. The corresponding relative cross section could be also estimated using $1 - \sigma'_{DEC} - \sigma'_{DP}$. It was found to vary from 80% (±2%) to 70% (±2%) with increasing charge $q$.

### B. Calculation of the electron capture distances and total cross sections

In HCl-atom collisions, the electron capture process can be described using the classical over-the-barrier model (COBM) [30] for two approaching point charges. The capture distance estimated with the COBM depends on the charge state of the projectile, the target ionization potential $I_p$, and the charge state of the target. The COBM has been modified and applied to larger systems such as C$_{60}$ [4] and polycyclic aromatic hydrocarbons [31]. Electron capture dynamics involving biomolecules such as cyt-C are even more complex. Unlike atomic targets or C$_{60}$, protonated cyt-C targets do not have a spherical symmetry and their conformation depends on the charge state of the molecule. Two examples of conformational changes are shown in Fig. 5. For high charge states (15+ to 19+), the molecule is expected to exist in a linear extended configuration [Fig. 5(b)], with the length estimated to be $L_p = 20.5$ nm = 388 a.u. In order to estimate the one-electron capture distance and to roughly estimate the total electron capture cross sections using COBM, we considered a simple model called in the following “model total cross section” in which the target molecular ion was represented by a linear segment of length $L_p$, along which $q$ positive point charges are distributed at equal distances. For a given collision geometry, we have calculated the electrostatic potential energy curve of an electron escaping from cyt-C $[M + qH]^q+$ to Xe$^{8+}$ for variable impact parameters. Interactions of the electron with the charge (8+) of the projectile, the charges (q+) distributed along the molecule, and the charge (1+) of the site left by the electron were taken into account. The so-called one-electron capture distance is obtained at a critical impact parameter where the binding energy of the electron in the electrostatic field of the projectile reaches the top of the potential curve. For collisions at shorter distance, the electron transfer from cyt-C $[M + qH]^q+$ to the approaching Xe$^{8+}$ ion can occur over the potential barrier.

In the following, the center of a molecular target is defined as the origin (O) of a coordinate system (Fig. 6). The z axis is defined by the direction of Xe$^{8+}$ projectile ion beam. We consider in the first step collision geometries [Fig. 6(a)] where the molecule is aligned along the x axis, hence perpendicular...
to the projectile trajectory. For this configuration, the impact parameter is given by \( R = (X,Y) \). We analyze first the cases where the electron transfer takes place near one of the ends of the molecule. To illustrate the method, we present a particular collision geometry, where the trajectory of the projectile is in the plane \( Oxz \) and the impact parameter is given by \((X,0)\) with \( X > R_p \). The potential energy curve of the escaping electron along the \( x \) axis between one of the ends of the molecule \((R_p,0)\) and the projectile \((X,0)\) was expressed as

\[
V(x) = - \sum_i \frac{1}{|x - R_i|} = \frac{8}{|X - x|} - \frac{1}{|X - R_p|},
\]

where \( R_i \) stand for the coordinates of protons along the molecular chain. The barrier of the potential energy curve \( V(x) \) was estimated for each impact parameter \( X \). It was compared with the binding energy of the electron attached to the end of the molecule in the electrostatic field of the projectile, \(-|I_p(q) + 8/|X - R_p|\). The ionization potential \( I_p(q) \) for protonated cyt-C was approximated using a linear relation, \( I_p(q) = 11.5 + 0.2q \) eV, obtained by extrapolation of the measurement of Giuliani et al. [26]. The capture distance \( X_c \), using target molecules with \( q \) varying from 15+ to 19+ has been calculated to be 214.6, 214.8, 215.0, 215.2, and 215.4 a.u., corresponding to distances with respect to the end of the molecule \( X_c = 17.6, 17.8, 18.0, 18.2, \) and 18.4 a.u., respectively. For other near-end collisions with impact parameters \((X,Y)\), \( X > R_p \), and \( Y \neq 0 \), comparable distances from the end of the molecules were found. Therefore, the geometrical cross section for the capture of an electron from one end of the molecule is given approximately by \( \pi/2X_c^2 \). Considering the two ends of the molecules, the total cross section for electron capture in near-end collisions is given by \( S_t = \pi X_c^2 \).

For collision geometries shown in Fig. 6(a), the projectiles pass most probably near the molecular chain with impact parameters \( |X| < R_p \), \( Y \neq 0 \). In such cases, the capture takes place preferentially from the closest amino acid. Along the cyt-C chain, the \( I_p \) to remove an electron is not constant. It depends on the specific amino acid from which the electron originates and its distance from the nearby protonated sites. We recall here that the protons are attached preferentially to the basic residues as arginine (R) and lysine (K). Although the tryptophan amino acid has the lowest \( I_p \) [32] (letter code W, \( I_p = 7.44 \) eV) of all amino acid constituents of cyt-C, it is not necessarily easier to remove an electron from the W site of a highly protonated molecule. Indeed, the W amino acid is close to the neighboring protonated sites and the electron binding energy from this W site could be larger than from some other amino acids. Williams et al. [21] have calculated the proton distribution versus the number of protons on cyt-C \([M + qH]^+\). From that work, one can notice that the amino acid sites with low \( I_p \) are those far away from the protonated sites, notably, around residue numbers 32, 45, and 63 [see Fig. 5(a)]. It can be expected that in collisions near these sites, the electron transfer may take place more easily leading to larger capture distance. In collisions close to other sites with slightly larger \( I_p \), the electron transfer is expected to occur at shorter distance. In the present crude model, the above features were not taken into account. The linear relation \( I_p(q) = 11.5 + 0.2q \) eV was used as an averaged value without discerning the amino acid or proton sites. The COBM was applied to the case where the projectile passes in the perpendicular bisecting plane between the two most central protons at impact parameter around \((0, Y)\). The electron transfer was estimated to occur at \((0, Y_c)\) at the capture distance \( Y_c(0) = 28.4, 29.2, 30.2, 31.0, \) and 31.8 a.u. for the charge \( q \) varying from 15+ to 19+, respectively. In order to estimate the electron-transfer cross section, the capture distance \( Y_c(X) \) should be estimated for other impact parameters, \((X, Y)\), \( -R_p < X < R_p \). As a rough approximation, it was considered as constant along the molecular chain \( Y_c(X) = Y_c(0) \), noted as \( Y_c \). Taking into account collisions at both sides of the molecule, the electron capture cross section \( S_{ch} \) along the chain was approximated as the surface area of a rectangle of width \( L_p \) (length of the molecule) and height \( 2Y_c \); \( S_{ch} = 2Y_cL_p \).

In the second step, we consider collision geometries as shown in Fig. 6(b). A random orientation of the molecular chain in the space is characterized by the angle \( \theta \) with respect to the \( z \) axis. The cross section for collisions along the molecular chain is reduced to \( S_{ch} \sin \theta \) while that for near-end collisions \( S_t \) can be considered as constant. Making the average over the \( 4\pi \) solid angle, the mean value \( <S_{ch}> \) is calculated by the integral, \( \frac{1}{4\pi} \int_0^{2\pi} \int_0^{\pi} S_{ch} \sin \theta \sin \theta d\theta d\phi \). The total cross section \( \sigma_{total} \) including \( <S_{ch}> \) and \( S_t \) was estimated by \( \sigma_{total} = \pi/4S_{ch} + S_t \). It slightly increases from \( 35 \times 10^{-14} \) cm\(^2\) to \( 40 \times 10^{-14} \) cm\(^2\) with the charge of protonated cyt-C varying from 15+ to 19+. The increase in the electron capture cross section with the charge of the molecule seems to be counterintuitive. Indeed, due to the Coulomb attraction from protons, it is obviously more difficult to remove an electron from a system with increasing positive charge. This is consistent with the linear increasing dependency of \( I_p \) on \( q \). However, this effect is counteracted by the presence of the HCl in the vicinity. In fact, the positive charges along the molecule reduces the potential barrier encountered by the escaping electron and furthermore, the higher the charge of the molecule, the lower the potential barrier.
in the end, the electrostatic field of the highly protonated molecule makes the electron-transfer process a bit easier. This leads to the increase of the over-the-barrier transfer distance with increasing target charge $q$ as evidenced in each of the above impact conditions. Comparing the two analyzed typical cases in impact geometries of Fig. 6(a) with $R = (X, 0)$ and $R = (0, Y)$, at the same collision distance ($X' = X - R_p = Y$) for the same charge $q$, the potential barrier was found lower for the second case. Indeed, the electrostatic field due to the $q$ protons is stronger along the charged molecular chain than at the end of it. This results in the increase of the capture distance in collisions along the molecular chain leading to $Y_c > X_c$ for the given $q$. The variation tendency of the model total cross section $\sigma_{\text{total}}$ with $q$ is in qualitative agreement with the measured increase in the parent ion depletion ratio $\rho$ from 17% ($\pm 1\%$) to 24% ($\pm 1\%$) for $q$ varying from 15+ to 19+. Using the model total cross section $\sigma_{\text{total}}$, the absolute cross sections for SEC and DEC processes were estimated using the measured relative cross sections, $\sigma_{\text{SEC}} = \sigma_{\text{total}} \sigma'_{\text{SEC}}$ and $\sigma_{\text{DEC}} = \sigma_{\text{total}} \sigma'_{\text{DEC}}$. In Fig. 7, we have plotted $\sigma_{\text{SEC}}$ and $\sigma_{\text{DEC}}$ versus the charge of cyt-C $[M + qH]^q+$. Both SEC and DEC cross sections are nearly constant as a function of the charge of the protein.

C. Deprotonation

In Fig. 4, the DP peaks observed at the nominal mass over charge close to the values expected for the intact molecular ions with a lowered charge, $q-1$, are attributed to the deprotonation process, i.e., the loss of a proton leading to $[M + (q-1)H]^{q-1+}$. The relative cross section of the DP peaks $\sigma'_{\text{DP}}$ were found to depend strongly on the initial charge of cyt-C $[M + qH]^q+$. Using the model total cross section $\sigma_{\text{total}}$, the absolute DP process cross section versus the initial charge $q$ has been estimated from the measured relative cross sections, $\sigma_{\text{DP}} = \sigma_{\text{total}} \sigma'_{\text{DP}}$ (see Fig. 7). To confirm the attribution of the DP peak to the deprotonation process, other possible mechanisms that may result in a decrease of the precursory parent ion charge state are considered and discussed below:

$$[M + qH]^{q+} + Xe^{8+} \rightarrow [M + qH]^{q-1+} + Xe^{9+}, \quad (2)$$
$$[M + qH]^{q+} + Xe^{8+} \rightarrow [M + qH]^{q+n+1} + Xe^{(8-n)+} \rightarrow [M + (q - n - 1)H]^{q-1+} + (n + 1)H^+ + Xe^{(8-n)+}. \quad (3)$$

The first reaction (2) corresponds to single electron transfer from the projectile to the highly protonated protein. Similar charge-decrease process via the capture of an electron by protonated proteins has been observed in collisions with anions [29]. However, in the present case, due to the large ionization potential of about 171 eV for Xe$^{8+}$, the transfer of a strongly bound electron from Xe$^{8+}$ to $[M + qH]^{q+}$ is highly improbable. Therefore this mechanism is eliminated in the interpretation of the DP peak. The second reaction (3) consists of two steps, i.e., the capture of $n$ ($n = 1$ or 2) electrons by the projectile, corresponding to SEC or DEC processes, followed by the loss of $n+1$ protons from the protein. Similar asymmetrical dissociation processes have been observed in collisions between highly charged ions and neutral targets as fullerene [33–35] clusters or clusters [36]. In the latter cases, multiple electron transfer may lead to the simultaneous excitation of the targets, which is followed by the statistical thermal emission of one or several small charged fragments. The asymmetrical fission of fullerene C$_{60}$ has been observed in collisions with Xe$^{8+}$ projectiles at 80 keV. However, comparing to the intact singly or multiply charged C$_{60}$, the fission yield amounted to only several percent of the total electron capture cross section. It was attributed to collisions at short impact parameters where a large amount of energy necessary to induce the fragmentation was deposited in the fullerene. Analogous to C$_{60}$, the loss of $n+1$ protons from $[M + qH]^{(q+n)+}$ should occur in closer collisions with much smaller cross sections than that of intact molecules. If the peak DP was the result of SEC [$n = 1$ in the reaction (3)] followed by the loss of 2H$^+$, its yield should be expected much smaller than that of the peak SEC. The measured values show, however, the contrary. In Fig. 7 one can see that the cross section $\sigma_{\text{DP}}$ is on the same order of magnitude as $\sigma_{\text{SEC}}$ and at $q = 18$ and 19, $\sigma_{\text{DP}}$ is even larger than $\sigma_{\text{SEC}}$. This very different charge variation tendency of $\sigma_{\text{DP}}$ from $\sigma_{\text{SEC}}$ is in favor of excluding the contribution of reaction (3) to the DP peak, although the fragmentation mechanism after a SEC process and its charge dependency is still unknown. Here, we attribute tentatively the DP peaks observed in Fig. 4 to the deprotonation process,

$$[M + qH]^{q+} + Xe^{8+} \rightarrow [M + (q - 1)H]^{q-1+} + Xe^{8+} + H^+. \quad (4)$$

The deprotonation process is tentatively interpreted as fast ejection of a proton during the collision. Fast ejection of atomic ions has been also observed with significant yield in collisions between Ar$^+$ at 7 keV and C$_{60}$ [37]. It was interpreted as due to the momentum transfer at short distance between the heavy incident Ar$^+$ and one of the carbon atoms in head-on collisions. The variation tendency of the measured C$^+$ yield with the projectile mass and velocity was in good agreement.
with the so-called nuclear stopping in collisions with matter. Nevertheless, such direct atomic knock-out type interaction is not in  

Indeed, short-distance interactions between the projectile ion and randomly one of the atoms of the target should be able to provoke prompt loss of other constituents leading to fragments such as $[M + q \text{H} - \text{C}]^{q+}$ or $[M + q \text{H} - \text{N}]^{q+}$. Obviously, these peaks are not observed in the mass spectra (Fig. 4). Additionally, the cross section for short-distance head-on collisions should be much smaller than that for the long-distance electron-transfer process and it should be sensitive to the kinetic energy of the collision rather than the charge of the collision partners. In the present experiment, we have observed the contrary. The measured DP cross section increases strongly with the charge of the molecular target and becomes even larger than the SEC cross section for $q = 18$ and 19 (Fig. 7). In order to analyze the role of the projectile charge, we have performed an experiment using Xe$^{5+}$ at 80 keV colliding on trapped cyt-C$[M + 16 \text{H}]^{16+}$. Although the kinetic energy of the projectile ion beam is equivalent, the yield of the DP peak was found to be significantly reduced in comparison to that obtained in collisions with Xe$^{8+}$. Hence, the DP peak is characterized by a large cross section and a strong dependency on both the charge of the target molecule and the charge of the projectile. These features suggest that the observed charge sensitive DP process seems to be driven by the long-distance Coulomb interaction between the multicharged incident ion and the protons and it occurs in long-distance collisions rather than in short-distance head-on collisions.

To remove a H$^+$ from a protonated molecule, the minimum energy cost is given by the apparent proton affinity ($A_p^{app}$) i.e., the apparent binding energy of the proton. For multiply protonated ions, the apparent binding energy of the proton at site $t$ is specified by ($A_p^{app}$)$_t$. Williams and co-workers [21] have proposed the following relation,

$$
(A_p^{app})_t = (A_p)_{\text{intrinsic},t} - \sum_{i=1, i\neq t}^q \frac{1}{R_{i,t}}.
$$

where the first term ($A_p^{\text{intrinsic},t}$) is the proton affinity of a molecule protonated at site $t$ (the $A_p$ of the basic site in the absence of other charges) and the second term corresponds to the decrease of the binding energy of this proton due to its interaction with the protons at all other sites. We estimated ($A_p^{app}$)$_t$ for two extreme conformational cases of a model cyt-C: a native structure (1CYC) based on the x-ray diffraction analysis taken from the protein data base pdb [38] and an extended structure built by setting the two dihedral angles on both sides of each peptide bond to 180°. Views of both obtained structures are shown in Fig. 5. As the peptide sequence of bovine cyt-C contains 20 basic functional groups (see Fig. 5) that can be readily protonated, i.e., 18 lysine (K) and two arginine (R) residues, these groups were considered as the most likely sites for positive charge localization. The distribution of the $q$ charges among these 20 possible sites ($2 \leq q \leq 20$) was then determined for each structure by minimizing the Coulomb energy, $\sum_{i=1}^q \sum_{j>i}^q \frac{1}{R_{i,j}}$, under the assumption that each proton from a basic residue carried a full +1 charge. For the minimized distribution, ($A_p^{app}$)$_t$ of protons at all occupied sites was calculated with Eq. (5) using a constant value ($A_p^{\text{intrinsic},t}$) = 10.4 eV, corresponding to the proton affinity of lysine residues [21]. The minimum value of ($A_p^{app}$) was defined as the apparent proton affinity of the molecule, $A_p^{app}$. The calculated value of $A_p^{app}$ is plotted in Fig. 8 as a function of $q$ for the two conformations. As expected, the extended linear conformation leads to higher $A_p^{app}$. The difference between $A_p^{app}$ of native and linear conformations increases from 0.2 to 4.7 eV as the charge $q$ increases from 2+ to 20+. For the native structure, on average, each additional charge lowers $A_p^{app}$ by ~0.6 eV. A negative $A_p^{app}$ value is reached for $q > 19$, which corresponds therefore to the maximum charge of this structure. For the extended structure, the decrease of $A_p^{app}$ with $q$ is less important and the value of $A_p^{app}$ remains positive over the whole charge range investigated. These modeled values are in good agreement with those reported by Williams and co-workers [21] using a similar point-charge approach. For the extended configuration, we note also an abrupt decrease of $A_p^{app}$ at charge 16+ and a larger slope in the high charge range. In fact, for $q \geq 16$, extra sites unoccupied at low charge state are involved in the protonation process, for example, the C terminal (103), the P residue (75) and some K residues (12, 54, and 86) (Fig. 5). Some of these sites are located very close to other adjacent protonation sites already filled. Interaction between protons at shorter distance leads to the decrease of ($A_p^{app}$)$_t$ of the engaged protonation sites. This explains the fast variation of $A_p^{app}$ with $q$ from 16+ to 19+.

The decrease of $A_p^{app}$ with increasing $q$ is in qualitative agreement with the measured variation tendency of $\sigma_{DP}$. Indeed, for molecules in higher charge states, less energy is needed to remove a proton. As a consequence, the proton loss can occur at larger collision distances leading to a larger DP cross section. The underlying mechanism for proton loss, however, is still unclear. A plausible explanation is the following: During the approach of the multicharged projectile, the electronic cloud of the target molecule, which ensures the binding of the nearest H$^+$, is most strongly polarized. This may lead to a temporary suppression of the binding “barrier” of the proton. As long as the duration for the barrier suppression is comparable to the period of H$^+$ oscillation, the proton might escape from the parent molecule during the collision.

![FIG. 8. (Color online) Calculated apparent proton affinity as a function of the charge of protonated cyt-C, $[M + q \text{H}]^{q+}$.](image-url)
IV. CONCLUSION

In summary, in collisions between Xe$^{8+}$ and cyt-C $[M + qH]^q +$ at 96 keV, for charge state from $q = 15$ to $19+$, the single and double electron capture (SEC and DEC) processes dominate and leave the protein intact. The cross sections for these processes remain nearly constant with the charge of the protonated cyt-C. The measured population depletion ratio due to ion impact increases with the charge of the molecule showing the same tendency as the electron capture cross section estimated with the over-the-barrier model. Unexpected peaks, $[M+(q-1)H]^q+1$, are observed with increasing cross section for $q$ varying from 15+ to 19+. These peaks are attributed to the deprotonation process, DP, i.e., the loss of a proton from the parents $[M + qH]^q +$. The variation of the measured cross section $\sigma_{dp}$ as a function of $q$ is in good accordance with the calculated proton affinities of cyt-C $[M + qH]^q +$ showing a monotonic decrease with increasing charge $q$. This DP process is tentatively interpreted as due to the temporary barrier suppression for the binding of a H$^+$ in long-distance interactions between the multicharged projectile and the binding electronic cloud. More experiments using projectile ions at different velocities and charge states would be needed in order to confirm the attribution of the deprotonation process and get more insight on the proton loss dynamics.


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Areal density effects on the blocking of 3-keV Ne\(^{7+}\) ions guided through nanocapillaries in polymers

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We studied blocking effects on ion guiding through nanocapillaries in highly insulating polymers. The experiments were initiated in view of a previous study with capillaries in polycarbonate (PC) for which strong blocking effects were observed, whereas for PET these effects could not be detected. The aim was to find out whether the different results are caused by differences in the PET and PC materials or by differences in the areal densities of the capillaries. Transmission experiments of 3-keV Ne\(^{7+}\) ions were performed for a variety of PET samples with an areal capillary density ranging from 3 to 60 \(\times 10^6\) cm\(^{-2}\). The tilt angles were close to zero degree because previous blocking effects were found to be largest at small angles. Our results clearly show that blocking effects also exist for PET and that they are sensitively dependent on the areal capillary density, i.e., the mean capillary distance. The potential produced by the charges accumulated in neighboring capillaries is calculated showing that it plays an important role in the ion blocking. In addition, model calculations are performed providing expressions to determine the direct ion transmission for small tilt angles and divergent ion beams.

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I. INTRODUCTION

In recent years the guiding of highly charged ions in capillaries with diameters of a few hundred nanometers has received considerable attention. Capillaries in highly insulating materials like polymers accumulate ions at the wall so that charge patches are created. Sufficient charge collection produces a repulsive electric field, which results in the deflection of subsequently passing ions. The deflection occurs at relatively large distances to the capillary wall so that electron capture into the projectile is inhibited. Thus, the ions are guided along the capillary axis maintaining their incident charge state during their passage even when the capillary axis is tilted with respect to the incident beam direction. The essential property of the ion guiding is the self-organizing process, which governs the charge deposition inside the capillaries [1].

Initial studies of ion guiding phenomena in insulating materials were performed using capillaries in polyethylene terephthalate (PET) [1–3]. During past years the interest in this field increased and several laboratories performed experiments using different materials such as PET [4–9], polycarbonate (PC) [10], SiO\(_2\) [11], and Al\(_2\)O\(_3\) [12–14]. Moreover, electrons were used as projectiles guided through capillaries in Al\(_2\)O\(_3\) [15,16] and PET [17,18].

In addition to multitude capillaries, guiding of highly charged ions were studied between flat glass plates [19] and in single capillaries with constant diameter [20,21]. In addition, single capillaries in tapered geometry were applied with the intention to produce submicrometer sized beams [22,23]. In a recent investigation the temperature of a single capillary was varied to control the influence of the surface conductivity [24]. Apart from the experimental work, a series of theoretical studies [25–29] has provided detailed information about the guiding mechanisms.

In previous experiments particular attention has been given to the dynamic properties of the ion fraction guided through the capillary [30–33]. Due to the formation of the entrance charge patch, the ion transmission starts delayed after a certain threshold and rises to a maximum where stationary (equilibrium) conditions are expected to be reached and maintained. Commonly one considers the system in equilibrium when the fraction of transmitted ions remains constant after reaching a maximum transmission. For instance, stationary conditions have always been observed in ion transmission experiments performed with PET. Only recently, experiments with PC capillaries have shown that after reaching a maximum the transmitted ion fraction decreases with increasing charge insertion [34]. This observation has been referred to as a blocking effect on the ion transmission.

In the previous study [34] it has been confirmed that the blocking effects are absent for PET capillaries even for a considerable amount of inserted charges. It should be noted, however, that the number of capillaries per unit area in PET was an order-of-magnitude smaller than in PC. (Throughout this work the number of capillaries per unit area will be referred to as areal capillary density or simply as capillary density.) The areal density of the PET capillaries was \(6 \times 10^6\) cm\(^{-2}\), whereas it was from 1 to \(5 \times 10^6\) cm\(^{-2}\) for PC capillaries [34]. Hence, the question arose whether the blocking effects are produced...
by the difference in the PC and PET material properties or by the difference in the areal capillary densities. Note that the capillary density determines the mean distance of neighboring capillaries.

To address this question, it is useful to first consider further information from previous studies. For instance, it was found that the blocking effect in PC capillaries increases with decreasing tilt angle \cite{34}, i.e., the blocking effect maximizes for untilted capillaries. This finding is unexpected as the amount of charge deposited into the capillary increases with tilt angle. However, for nonzero tilt angle the charge is accumulated primarily in the entrance charge patch, whereas the charge deposited in the center of the capillary is relatively small \cite{29}. It appears that the charge in the center of the capillary has an important effect on the ion blocking.

In recent simulations \cite{28} it was concluded that the charge accumulated in a single capillary is not sufficient to create a repulsive field strong enough to reject the incident ions. This finding suggests that ion blocking is enhanced by the multitude of capillaries present in the neighborhood. Such collective neighbor effects were considered in simulations of ion guiding \cite{25}. Experimentally, the neighbor effect is expected to be enhanced, when the distance to the capillary neighbors is reduced by increasing their areal density.

In the present work, we investigate the fraction of transmitted ions for capillary densities varying by more than one order of magnitude. The experiments are performed with PET capillaries to find out whether blocking effects exist also for this material. The data are acquired for tilt angles close to zero degree, since the blocking is largest for untilted capillaries. The experiments confirm that the blocking effect is strongly influenced by the mean distance of the capillaries. The ion blocking is attributed to the collective field produced by the charge accumulation in neighbor capillaries.

**II. EXPERIMENTAL METHOD**

The experiments were performed in an ultrahigh vacuum chamber mounted at the 14-GHz electron cyclotron resonance (ECR) ion source of the distributed LEIF-Infrastructure ZERNIKE-LEIF at KVI Groningen (Netherlands) \cite{35}. The experimental method has been described in detail before \cite{32} so that only a few details are pointed out here. The vacuum chamber was used in a high-vacuum mode, i.e., the base pressure was a few 10^{-8} mbar. The apparatus was set on high voltage to allow for the deceleration of the incident Ne^{7+} ions from 49 keV down to 3 keV. The ion current was varied within the range from 10 to 3000 pA. The beam was collimated to a diameter of 1.3 ± 0.2 mm with a divergence of about ±0.25°.

Cylindrical parallel oriented capillaries were prepared by irradiating 10 ± 1 μm PET foils with 2.2-GeV gold ions at the GSI Helmholtzzentrum in Darmstadt \cite{36}. The applied ion fluence was varied by a factor of 20 between 3 × 10^6 and 6 × 10^7 ions/cm². The irradiated foils were etched in 6N NaOH, converting each ion track into a cylindrical capillary. The duration of the etching was controlled such that the diameter of the capillaries was 140 ± 15 nm. A gold layer was evaporated in four directions under 30° on the front and the back sides of the PET foils forming a film of ∼20-nm thickness to avoid a macroscopic charge build up mainly at the sample front surfaces. Figure 1 presents images obtained with scanning electron microscopy (SEM) to show the areal density \(\delta_c\) of the capillaries. Due to the stochastic nature of the ion beam the capillary entries are statistically distributed over the surface. The densities and mean distances of the PET capillaries are summarized in Table I.

The capillary foils were mounted at a goniometer, which allowed for tilting the capillaries relative to the incident beam in two directions specified by the angles \(\psi\) and \(\varphi\). The tilt angle \(\psi\) is an important parameter in the present study. The azimuthal angle \(\varphi\) was kept constant after its zero value was fixed using a laser for alignment. The PET target foils were spanned into circular frames with an inner diameter of 7 mm. By shifting the frame with the goniometer, the incident Ne^{7+} ion beam could be positioned at different spots within the target area. Hence, the results described below were obtained with unirradiated capillaries if not otherwise stated.

The neon ions emerging from the capillaries were analyzed with respect to their charge state, energy, and emission angle using an electrostatic 180° spectrometer, which could be rotated by an angle \(\theta\) in the same plane as the tilt angle \(\psi\). In the experiments the angular distribution of the transmitted ions was measured with a position sensitive detector. The angular resolution of the spectrometer was around 0.1°.

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### Table I. Areal density \(\delta_c\) and mean distance \(l_c\) of the PET capillaries used in this work. For all samples the capillary diameter is 140 nm and the length is 10 μm.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\delta_c) (cm²)</th>
<th>(l_c) (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3 × 10^6</td>
<td>6</td>
</tr>
<tr>
<td>b</td>
<td>5 × 10^6</td>
<td>4.5</td>
</tr>
<tr>
<td>c</td>
<td>3 × 10^7</td>
<td>2</td>
</tr>
<tr>
<td>d</td>
<td>6 × 10^7</td>
<td>1</td>
</tr>
</tbody>
</table>
Ne$^{7+}$ ions was measured for tilt angles near 0°. The angular resolution of the analyzer was 0.2° full width at half maximum (FWHM). For the direct incident beam (without the capillary target) a Gaussian-like angular profile with a FWHM of 0.5° was acquired. This width is expected to be a composition of the beam diameter, its divergence, and the angular resolution of the analyzer.

III. EXPERIMENTAL RESULTS

A. Transmission profiles

In the following, we present transmission profiles of 3-keV Ne$^{7+}$ ions, whose incident charge state was preserved during the passage through the capillary. A transmission profile represents an angular distribution $\frac{dY}{d\Omega}$ of Ne$^{7+}$ ions transmitted through the capillaries as a function of the observation (or emission) angle $\phi$. We recall that $\phi = 0°$ was fixed in the experiments. Figure 2 shows a series of selected transmission profiles for the tilt angles of $\psi \approx 0°$. The columns from left to right show profiles obtained with increasing capillary density (values given in the upper panels).

Each transmission profile is normalized to an integral current of $Q_d = 1$ nC, where $Q_d$ is the total charge deposited by the beam on the front surface of the target foil. For a given incident beam current the deposited charge is a measure of time. Each graph indicates the charge $Q_d$ collected until the instant when the profile is measured. Hence, the results in a given column represent the temporal evolution of the transmission profile for the corresponding capillary density.

From Fig. 2 it is seen that the peak position of the transmission profiles is rather constant with increasing charge deposition, i.e., there are only small deviations of the profile position from the center value represented by the vertical dashed line. In Fig. 3 the peak positions are shown as open circles representing the mean emission angle obtained by numerical integration $\theta = \int \theta (dY/d\Omega)d\theta/\int (dY/d\Omega)d\theta$ [32]. The data are plotted as a function of the charge $Q_{in}$ inserted into a single capillary, which will be used in the following instead of the deposited charge $Q_d$ (Fig. 2). The charges $Q_{in}$ and $Q_d$ are related by $Q_{in} = Q_d(d/D)^2$ where $d$ is the diameter of the capillary (140 nm) and $D$ is the diameter of the ion beam (1.3 mm). More information about the integration procedure and the charge conversion is given in a recent study [37].

From Fig. 3 it is seen that the mean emission angle changes slightly when the charge insertion increases. The variation of the mean angle reveals the formation of transient charge patches as has been discussed in detail previously [30–33]. Transient charge patches can be produced when the tilt angle deviates from zero. It should be pointed out that due to experimental uncertainties, it is difficult to set the capillary tilt angle exactly to zero. The tilt angle can only accurately be determined by the analysis of the data after the experiment. The actual tilt angle can be observed after sufficient charge is inserted into the capillary (i.e., asymptotically after reaching equilibrium conditions) since asymptotically the transient charge patches have essentially disappeared. In Fig. 2 the asymptotic peak positions vary within ±0.3°, which may be considered as the experimental uncertainty of setting the capillary tilt angle.

Moreover, from the experimental analysis the full width at half maximum $\sigma_{\theta}$ of the transmission profiles can be
determined. In Fig. 3 the profile widths are plotted as curves represented by full circles. The curves show that widths are first increasing with increasing charge insertion and approach stable values for further charge insertion. We note that for the present tilt angles close to 0° the width \( \sigma_\theta \) varies between 1° and 2° which is smaller than the width observed for nonzero tilt angles, as summarized in previous scaling laws [6]. Also, it is noted that the width of the profiles essentially does not change when the areal density of the capillaries is varied.

Diverse effects are responsible for the width of the transmission profiles, which have been discussed previously [6,25]. One may suppose that the nonparallelism of the capillaries produces the dominant contribution to the width of the emission profiles. However, this supposition is incorrect since under guiding conditions the nonparallelism results in a width independent of the charge state and energy of the projectile, which is clearly against experimental observations [6].

Returning to Fig. 2 we note that the transmission profiles vary in intensity when the charge deposition increases. Each profile is multiplied by a factor, which reveals its intensity variation. In the first column the multiplication factor is always 20 indicating that the profile intensity is essentially constant. However, in the last column the multiplication factor changes from 1 to 20 indicating a significant intensity loss with increasing charge deposition. This loss of intensity is a signature for the ion blocking [34]. Hence, we may conclude that blocking effects also exist for capillaries in PET so that there is no principle difference between the capillaries in PET and PC.

**B. Recovery of the ion transmission**

In the following, we address the question whether the loss of the ion transmission is transient or permanent. In principle the loss of transmission could be permanent due to fundamental material changes in the capillary interior. Thus, we consider the total ion yield \( Y_t \) transmitted through the capillaries, which is obtained from the integration of the transmission profiles,

\[
Y_t = \int \frac{dY(\theta, \phi)}{d\Omega} d\Omega. \tag{1}
\]

Since in this work only the ion yield with respect to the angle \( \theta \) was measured, the integration over the angle \( \phi \) was performed assuming that its dependence is described by a Gaussian function. The width \( \sigma_\phi \) of the Gaussian was set to be equal to the width \( \sigma_\phi \) of the transmission profile obtained for sufficiently large charge deposition. Then, the total yield was obtained by numerical integration of the experimental data.

As mentioned, we may consider the possibility that the loss of transmission is permanent. The ions could be blocked by an enhanced collection of charges inserted into the capillaries. In the case that the deposited charges retain a certain mobility, it is expected that after some time the charges are removed and the capillaries become again transparent. On the other hand, it may be possible that due to specific material properties the capillaries remain charged so that they permanently inhibit the passage of the ions.

To find an answer to these questions, the ion transmission at a fresh spot on the capillary sample was repeated after a

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**FIG. 4.** Recovery of the capillary transmission after a 15 hour pause. The data were measured using 3-keV \( \text{Ne}^{+} \) ions transmitted through PET capillaries with a density of \( 6 \times 10^7 \text{ cm}^{-2} \). In (a) the ion transmission for a fresh spot on the capillary sample is shown. In (b) the repetition of the experiments at the same spot is presented. The data are plotted as a function of the inserted charge \( Q \).

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15 hours pause with the same capillaries as used before. After this pause it is expected that the majority of mobile charges are removed from the capillary interior. The measurements were performed with the highest available capillary density of \( 6 \times 10^7 \text{ cm}^{-2} \) (Table I) to enhance the blocking effect.

In Fig. 4 the total intensity of the transmitted ions is plotted as a function of the inserted charge \( Q \). Figures 4(a) and 4(b) show the results obtained with the fresh and used capillaries, respectively. (We recall that the transmission profiles associated with the fresh capillary measurement are shown in the last columns of Fig. 2.) For the fresh capillaries the ion intensity rises initially very rapidly to a maximum from where it drops continuously by more than an order of magnitude. The initial increase of the ion intensity is expected to be due to a guiding effect that compensates the intensity loss due to a small nonzero tilt angle. This intensity loss can be understood from Fig. 10(a) presented in the Appendix.

In Fig. 4(b) the repetition of the measurement shows the same qualitative behavior as the original experiment. After an initial rapid increase, the ion intensity decreases due to blocking effects. The similarity of the two curves in Figs. 4(a) and 4(b) indicates that the transmission properties of the capillaries are essentially recovered after the 15 hours pause. This shows that most of the charges within the capillary interior are removed. However, certain differences between the two curves persist revealing that some rest charge remained within the capillaries. Nevertheless, it may be concluded that the differences between PET and PC [34] are not caused by differences by the material properties. Thus, it is likely that the blocking is influenced by the capillary density. The role of the capillary density for the ion transmission will be analyzed next.
C. Capillary density effects on the blocking of 3-keV Ne$^7^+$ ions

In the following we study the temporal evolution of 3-keV Ne$^7^+$ ion transmission for different capillary densities. As before, the total ion yield $Y_t$ was determined by integrating the transmission profile partially shown in Fig. 2. However, for a better comparison of the data obtained with different capillary densities, we normalized the total intensity of transmitted ions. In the following, we consider the fraction of transmitted ions obtained by dividing $Y_t$ by the total number of ions $Y_m$ incident into the multitude of capillaries involved:

$$f_t = \frac{Y_t}{Y_m}.$$  \hspace{1cm} (2)

The number of incident ions $Y_m \propto \delta_c$ so that $f_t$ contains a division by that density. We note that the quantities $Y_t$ and $Y_m$ were measured only on a relative scale so that the fraction $f_t$ depends on a proportionality factor. However, the results for each capillary density involve the same proportionality factor, which will be determined below in comparison with the model calculations performed in the Appendix.

In Fig. 5 the results for the transmitted ion fraction are plotted as a function of the inserted charge. Each graph labeled (a)–(d) is associated with a certain capillary density in accordance with the notation in Table I. All curves exhibit a rapid increase at the beginning of the charge insertion, which can be attributed to small deviation from the $0^\circ$ tilt angle as already discussed for the data in Fig. 5(d) in the preceding section. As mentioned, the guiding effect compensates the initial loss of the transmitted ions.

After the guiding has reached equilibrium, we expect that for small angles the transmitted ion fraction reaches a maximum, which is nearly unity. However, the divergence of the beam leads to some loss of ions within the capillaries. For the present divergence of $\pm 0.25^\circ$ the transmitted ion fraction was determined to be 0.7 as a result of calculations in the Appendix. Thus, the unknown proportionality factor involved in $f_t$ was fixed by setting the maximum of the data in Fig. 5(a) equal to 0.7. Then, the curves for the other densities could be put on an absolute scale, too.

From Figs. 5(a) and 5(b) it is seen that the transmitted ion fraction for the lower densities of $3 \times 10^6$ and $5 \times 10^6$ cm$^{-2}$ are rather constant, whereas the results in Figs. 5(c) and 5(d) for the higher densities of $3 \times 10^7$ and $6 \times 10^7$ cm$^{-2}$ decrease strongly with the inserted charge. Hence, it is obvious that the observed blocking effects are much stronger for the high densities than for the low densities.

The strong decrease of the ion transmission can be understood in terms of a self-enhancing effect involved in the ion blocking process. Ion blocking is the result of a repelling field produced by the charge deposited in the capillary interior. This quantity will be denoted as an absorbed charge in the following. When the capillary density increases, the repelling field is enhanced by neighbor contributions. Then, the ion blocking is enhanced so that the charge absorbed within the capillaries increases, which in turn increases the repelling field and so on. Hence, we expect blocking effects beyond proportionality when the capillary density increases. The reason for the nearly instant onset of the ion blocking for the higher density capillary will be discussed in more details below in conjunction with model calculations.

The self-enhancing effect of the ion blocking can be revealed in more detail by means of the amount of charge $Q_a$ absorbed in the capillary interior. This charge is obtained from the absorbed charge current,

$$J_a = J_m - J_t,$$  \hspace{1cm} (3)

where $J_m$ and $J_t$ are the incident and transmitted current, respectively. The absorbed charge is deduced by the time integration,

$$Q_a(t) = \int_0^t J_a(t') \, dt',$$  \hspace{1cm} (4)

which can be replaced by the integration over the incident charge using $t = Q_m/j_m$ (and $dt = dQ_m/j_m$),

$$Q_a(Q_m) = \int_0^{Q_m} f_a(Q'_m) \, dQ'_m,$$  \hspace{1cm} (5)

where we introduced the current fraction $f_a = J_a/J_m = 1 - f_t$ obtained from the transmitted fraction $f_t$ given in Fig. 5.

The results of the integration are plotted in Fig. 6 where the absorbed charge $Q_a$ is given as a function of the inserted charge $Q_m$. The figure shows four curves each of which is attributed to a density of the capillaries. For the two lower capillary densities ($3 \times 10^6$ and $5 \times 10^6$ cm$^{-2}$) the absorbed charge amounts to about 30% of the inserted charge for values smaller than $\sim 35$ fC. However, for the higher capillary densities ($3 \times 10^7$ and $6 \times 10^7$ cm$^{-2}$) the absorbed charge amounts to values as large as 80% of the inserted charge. This large value for the absorbed charge is the reason for the self-enhanced blocking
process. Indeed, this is likely due to the blocking effects observed in Figs. 5(c) and 5(d) which are much stronger than those found in Figs. 5(a) and 5(b).

It should be pointed out that the absorbed charge stays only transiently within the capillary. Electric fields, produced by the absorbed charges, transport these charges out of the capillary interior to the exits where they are finally depleted at the gold film covering the sample surfaces [28]. Therefore the absorbed charge splits into two components:

\[ Q_a = Q_d + Q_c, \]

where \( Q_d \) is the charge depleted at the sample surface and \( Q_c \) is the collected charge remaining within the capillary. The electric field increases with increasing charge absorption so that the charge depletion increases, too. Therefore, the actual charge \( Q_i \) collected in the capillary is smaller than \( Q_a \). The latter charge can be used as a reasonable estimate for the collected charge only in cases for which the charge transport is small. Otherwise, \( Q_a \) has to be used as an upper limit of the actual charge present in the capillary.

IV. ION BLOCKING BY NEIGHBOR CAPILLARIES

In this section we describe a model that estimates the potential produced by neighbor capillaries leading to a partial or full blocking of the inserted ions. To obtain the potential produced by all charges, their Coulomb potentials are summed as follows:

\[ V(r) = \sum_i \frac{q_i}{|r - r_i|} + \sum_n \sum_i \frac{q_{i,n}}{|r + r_{i,n}|} + \text{images}, \]

where the first and second term refer to the given capillary and neighbor capillaries, respectively. The index \( i \) runs over the charges in a single capillary and the index \( n \) runs over the number of neighbor capillaries. The number of capillary neighbors labeled \( n \) was limited to a finite value beyond which more capillaries could be added without a noticeable change of the potential.

Within the present model, capillaries are assumed to have a square opening with size \( a \) and length \( L \). The square shape is chosen to achieve an analytic expression for the potential of a rectangular, homogeneously charged surface of the capillary interior. The summation over \( i \) in Eq. (7) is replaced by an integration, which can be solved analytically. The width of the opening is set to be \( a = d/\sqrt{2} \) so that the entrance areas of the square and circular openings are equal.

In Eq. (7) the term denoted images is added to account for the grounded metal layer evaporated on the front and back side of the PET foil (noted in the experimental Sec. II). The grounded metal layers are taken into account by adding a reasonable set of image charges (in fact, images of the charged surfaces) with alternating sign on both sides, using the metal layer as a mirror. More details will be presented in a future publication.

The replacement of the circular capillary by a square capillary is expected to be a reasonable approximation as the average distance between neighbor capillaries is significantly larger than the capillary diameter. Note that this simple model provides the potential inside a capillary due to an arrangement of charged rectangular surfaces in it, and a large ensemble of neighboring capillaries identically charged. However, it does not account for how these charge arrangements are created.

At the simplest arrangement, the deposited charge is assumed to be distributed homogeneously within the capillary. Moreover, only the upper and lower wall of the four capillary sides are covered by charges. The resulting potential for a
AREAL DENSITY EFFECTS ON THE BLOCKING OF 3-... PHYSICAL REVIEW A 88, 032902 (2013)

Figure 7 shows potentials from the model calculations for the capillary densities used in the experimental work (Table I). The potentials are given at the capillary center along its length \( L \). The potential labeled \( s \) refers to a single capillary. The charge of 35 fC, inserted into the capillary, is converted to absorbed charges using the curves in Fig. 6. The horizontal dashed line represents the potential barrier \( T_p/q = 428 \text{ V} \) which cannot be overcome by ions incident with kinetic energy of \( T_p = 3 \text{ keV} \) and charge \( q = 7 \). It is seen that the potentials for the lower capillary densities (\( 3 \times 10^6 \) and \( 5 \times 10^6 \text{ cm}^{-2} \)) are smaller than this barrier, whereas in the middle of the capillary, the potentials for the higher capillary densities (\( 3 \times 10^7 \) and \( 6 \times 10^7 \text{ cm}^{-2} \)) are larger than this barrier. Hence, for the high density samples the potential is dominated by the neighbors, while in case of the low density samples the potential is only slightly modified by the neighbors.

Further results are calculated for the maximum potential located in the middle of the capillary at 5 \( \mu \text{m} \). In Fig. 8 the data are plotted as a function of the inserted charge which is again converted to the absorbed charge by means of Fig. 6. The figure shows a dashed dotted line at 45 V, which crosses the curves labeled d, c, b, and a near the inserted charges 2, 4, 28, and 37 fC, respectively. Looking back at Fig. 5 one can see that the transmission starts to drop at \( \sim 1, \sim 2, 25, \) and 33 fC, respectively. It is likely that at the potential of 20–40 V the deceleration of the incident beam leads to an increase of the beam divergence which in turn increases the ion absorption at the capillary wall. Thus, the present model calculation explains the much faster ion blocking within the capillaries of higher densities observed in Fig. 5.

In addition, Fig. 8 shows that the curves associated with the lower densities do not cross the potential barrier indicated by the horizontal dashed line at 428 V. This is in accordance with the experimental results that the ion blocking is small for these densities. However, the curves for the higher densities cross the potential barrier at inserted charges higher than \( \sim 15 \text{ fC} \). Consequently, the ion blocking is found to be significant for these densities [see Figs. 5(c) and 5(d)].

The finding that the potential exceeds the barrier at 428 V should lead to a total suppression of the ion transmission through the capillaries. In fact, a potential larger than 428 V cannot be created by 3-keV Ne\(^{7+}\) ions so that the model is not applicable in this case. Rather, Figs. 5(c) and 5(d) show a non-zero transmission even for large charge insertion. There are two reasons for this observation. First, the present model is performed with a constant current density of the incident ion beam. However, the ion beam is expected to have a density distribution described by a Gaussian function with a finite width. At the edges of the ion beam the neighboring potential is reduced so that it may become lower than the potential barrier. This would lead to a partial transmission of the ions through the capillary.

Second, as indicated by Eq. (6) in the previous subsection, the absorbed charge \( Q_a \) should be regarded as an upper limit for the charge really present within the capillaries. This absorbed charge is reduced by the transport of the charge \( Q_d \) to the capillary exits, where they can be depleted at the grounded metal layer. In fact, the electric field directed along the capillary axis, responsible for the charge transport, can be deduced by differentiating the corresponding curves in Fig. 7. For the higher densities the longitudinal field exceeds the value of 0.1 V/\( \mu \text{m} \) in the vicinity of the capillary exits. This number is close to the electrical breakthrough value for PET [28]. Hence, for the high capillary densities, the absorbed charge is transported with a high probability to the grounded metal layers. The removal of the absorbed charge becomes relatively large for the highest capillary density so that it may be understood that the blocking effect for the density of \( 6 \times 10^7 \text{ cm}^{-2} \) is not much higher than that for \( 3 \times 10^7 \text{ cm}^{-2} \).

In particular, we note that in Figs. 7 and 8 the results labeled \( c \) and \( d \) are upper limits for the potentials. On the other hand, it is expected that the curves labeled \( a \) and \( b \) corresponding to the lower densities, represent realistic values. Altogether, the present model shows at least qualitatively that neighbor effects may be weak for capillaries of densities smaller than \( 10^7 \text{ cm}^{-2} \). However, they are important for capillary densities larger than this critical value.

V. CONCLUSIONS

The transmission of 3-keV Ne\(^{7+}\) ions through PET capillaries was measured to study blocking effects on the ion transmission for different capillary densities. The work was motivated by recent observations that PC capillaries exhibit strong ion blocking effects whereas these effects were absent for capillaries in PET [34]. Since the PC samples contained capillaries at a higher density than for the PET samples, the question arose whether the differences between PC and PET are produced by the different material properties or different densities.
The experiments clearly revealed that there is no principle difference between the PC and PET materials. Rather, the ion transmission is strongly influenced by the capillary density. For the lowest density of $3 \times 10^6$ cm$^{-2}$ the ion transmission was found to be practically constant in agreement with the previous results obtained with PET capillaries at the same density [34]. Thus, these low-density samples show no blocking. Similarly, the blocking effect is marginal for the PET samples with a capillary density of $5 \times 10^6$ cm$^{-2}$.

However, drastic blocking effects on the ion transmission are observed for PET capillaries with densities higher than $10^7$ cm$^{-2}$. Hence, the loss of transmission becomes increasingly important for a mean capillary distance of 2 μm and below (Table I). It appears that blocking becomes significant when the capillary half length $L$ is smaller than the capillary half length $l$. The significant increase of the ion blocking can qualitatively be explained by a self-enhancing process involved in the ion absorption within the capillary. The blocking leads to an increased absorption of incident charge in the capillary interior which, in turn, increases the potential responsible for the blocking and so on. It should be added that the absorbed charge is removed by the longitudinal field determined by the derivative of the potential. When the potential or field increases, more charges are removed so that the self-enhancing process is weakened. Therefore, it may be understood that the blocking effect for the density of $6 \times 10^6$ cm$^{-2}$ is not much higher than that for $3 \times 10^6$ cm$^{-2}$.

In view of the strong losses observed in the ion transmission the question arises why blocking effects in PET capillaries have not been reported in earlier work. We found here that the capillary density is an important parameter, which governs ion blocking. However, it cannot be excluded that other parameters are important as well. In particular, we expect that the conductivity of the inner capillary wall plays a certain role. Further work is needed to inquire about the material properties influencing the ion transmission at high capillary densities.

Finally, in model calculations it was shown that the ion blocking may strongly be influenced by capillary neighbors. In accordance with the experimental results it was shown that for smaller capillary densities the blocking effects are relatively small. On the other hand, blocking effects are found to be significant for higher densities also in agreement with the experimental results. In fact, the calculations overestimate the blocking since the charge transport to capillary exits was not included in the model. A more realistic modeling could be performed by incorporating the neighbor capillaries into simulations of the ion guiding [25]. In particular, future studies are needed for tilt angles of a few degrees, which were not treated in the present work.

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APPENDIX: MODELS FOR DIRECT ION TRANSMISSION

In this Appendix, we perform model calculations to analyze the direct ion transmission through a capillary, i.e., for ions that follow straight-line trajectories within a field-free capillary. As noted the experiments deal with ion transmission through capillaries with tilt angles of nearly zero degree. The direct transmission of the ion beam is strongly affected by any deviation from zero degree. Moreover, even if the tilt angle is exactly equal to zero, some intensity is absorbed within the capillary due to the fact that the ion beam slightly diverges. Here, we provide simple expressions, which allow for evaluating the ion transmission through a slightly tilted capillary involving a small beam divergence.

As noted before, the fraction of transmitted ions is given by $f_t = J_t/J_0$, where $J_0$ is the current, which enters into the capillary entrance and $J_t$ is the transmitted current leaving the capillary exit. The capillary has a length $L$ and a diameter $d$ as shown in Fig. 9. An important quantity is the aspect ratio $L/d$ defining the angle $\phi_0$ by $\tan \phi_0 = d/L$.

First, we consider a beam originating from a small source area $s$ at a distance $l$ from the capillary (Fig. 9). The current ejected from the source into the solid angle $\Omega$ is given by $J_s = j_s \Omega$, where $j_s$ is the current density characteristic for the source. The solid angle is obtained as $\Omega = \sigma/l^2$ where $\sigma$ is the area limiting the beam. The beam entering into the capillary is limited by the capillary opening $\sigma_0 = \pi d^2/4$. The transmitted beam is determined by the overlap $\sigma_c$ of two circles representing the entrance and exit opening of the capillary seen by an observer at the source point. The circles are displaced by the distance $y_m = L \tan \psi$ (Fig. 9).

Thus, the fraction of ions transmitted through the tilted capillary is obtained as $f_\psi = \sigma_c/\sigma_0$. The overlap $\sigma_c$ can be determined in closed form [29] yielding an expression, which depends only on the normalized quantity $\varrho = y_m/d = \tan \psi/\tan \phi_0$. For $d \ll L$ one may set $\varrho \approx \psi/\phi_0$, which is referred to as the relative tilt angle. In Fig. 10(a) the exact results for transmitted ion fraction $f_\psi$ is shown as a function of relative tilt angle $\varrho$. As expected, the effective opening is equal to unity for ion impact along the capillary axis and tends to zero when the tilt angle approaches the capillary aspect angle (for $\varrho \to 1$). It is noted that the transmission drops to 1/2 at the relative angle of $\varrho = 0.4$.

As shown previously [29], the exact transmission function can be approximated by the simple empirical expression,

$$f_\psi = (1 - \varrho)^\chi,$$

(A1)

for $\varrho \leq 1$ and $f_\psi = 0$ elsewhere, where $\chi = 1.38$. In Fig. 10(a) the approximate results from Eq. (A1) are shown as a dashed line. The two curves coincide very well.

![FIG. 9. Schematic drawing for the transmission of a direct beam through a slightly tilted capillary.](image-url)
AREAL DENSITY EFFECTS ON THE BLOCKING OF 3- . . .

PHYSICAL REVIEW A 88, 032902 (2013)

FIG. 10. Exact and approximate results for the transmitted ion fraction represented by a solid and dashed line, respectively. In (a) the transmitted ion fraction is given as a function of the relative tilt angle $\varphi$ and in (b) the transmitted ion fraction is given as a function of the relative divergence angle $\varphi_d$.

Next, we assume that the incident beam is absorbed within the capillary due to the divergence of the ion beam. It is characterized by the divergence angle $\psi_d$ assuming that most of the ions are emitted within $\pm \psi_d$. Here, an untitled capillary is considered, i.e., the capillary axis lies in the center of an extended source area governed by the beam diameter ($\sim$1.3 mm). Small fractions of the ion beam are emitted from ring elements with the area $\Delta s = 2\pi r \Delta r$ where $r = l \tan \psi$ determines the tilt angle $\psi$ (Fig. 9). As above, the relative tilt angle $\varphi \approx \psi/\phi_a$ is used. Moreover, we introduce the relative divergence angle $\varphi_d = \tan \psi_d/\tan \phi_a \approx \psi_d/\phi_a$.

The fraction $f_d$ of ions transmitted by a diverging beam is obtained by integration over the ring elements or, equivalently, over $\varphi$,

$$f_d = \int_0^1 f_\psi (\varphi) j_\psi (\varphi, \varphi_d) \varphi d\varphi.$$  \hspace{1cm} (A2)

where $\int j_\psi (\varphi) \varphi d\varphi = 1$. The upper limit of the integration is unity, since $f_\psi (\varphi) = 0$ for $\varphi > 1$ [Eq. (A1)]. The transmitted ion fraction $f_d$ is evaluated numerically using both a Gaussian function and a step function for $j_\psi$ with the full width $w_d = 2\psi_d$, which yielded similar results. The numerical results from the Gaussian function are represented by the solid line in Fig. 10(b) plotted as a function of the relative divergence angle $\varphi_d$.

The numerical results can be approximated by the expression,

$$f_d = \frac{3}{4} (1 - \varphi_d)^2 + \frac{1}{\pi}.$$ \hspace{1cm} (A3)

for $\varphi_d \leq 1$ and $f_d = 1/(4\varphi_d^2)$ elsewhere. Again, $\chi = 1.38$. The results from Eq. (A3) are given as a dashed line in Fig. 10(b). The agreement between the original and approximate expressions is very good.

As an example, for the present capillary experiments $\psi_d = \pm 0.25^\circ$ and $\phi_a = \arctan(0.014) = 0.8^\circ$ so that $\varphi_d = 0.31$. For this case Eq. (A3) yields a transmission of $f_d = 0.7$ corresponding to 30% of ions absorbed within the capillary. This number is in accordance with the result of a previous simulation [29]. We recall that this value for $f_d$ was used to normalize the fractions presented in Fig. 5.

To combine the ion transmission by the nonzero tilt and divergence angle we set

$$f_t \approx f_\psi f_d,$$ \hspace{1cm} (A4)

which is a reasonable approximation for $f_t \lesssim 1$.

Fragmentation of protonated oligonucleotides by energetic photons and \( \text{C}^{+}+ \) ions

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The ionization and fragmentation of trapped protonated dGCAT oligonucleotides upon interaction with energetic photons \((h\nu = 10–570 \text{ eV})\) and keV \( \text{C}^{+}+ \) ions was investigated by means of time-of-flight mass spectrometry. The observed fragmentation patterns are dominated by protonated and nonprotonated nucleobase ions and fragments of the deoxyribose moiety. Fragments exceeding the size of nucleosides are almost completely absent. Absorption of VUV photons as well as interaction with keV ions predominantly involves ionization or excitation of molecular valence electrons and accordingly the observed fragmentation patterns exhibit qualitatively similar features. Soft-x-ray-induced ionization of core level electrons accompanied by subsequent emission of an Auger electron shifts the fragment distributions towards smaller masses. This systematic study allows for insights into differences and similarities between ion- and photon-induced excitation and fragmentation mechanisms. In particular, the crucial role of the deoxyribose moiety for radiation-induced DNA damage that was predicted on the basis of gas-phase experiments using isolated deoxyribose molecules is confirmed.

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I. INTRODUCTION

Most biological effects of ionizing radiation are triggered by direct and indirect DNA damage. The latter is mainly due to DNA interaction with free radicals from radiolysis of nearby water molecules. Direct damage to DNA is induced either by the primary quanta of radiation themselves or by secondary particles such as electrons and ions. A typical experimental approach to assess direct and indirect effects is the irradiation of plasmid DNA in solution with x rays or fast ions and subsequent detection of single or double strand breaks [1–3]. Indirect damage can be quenched to a large extent by the addition of radical scavengers [3–5]. Low-energy electrons and (sub-)keV ions have a very short penetration depth and, therefore, their potential to induce strand breaks is most often studied using plasmid DNA deposited onto surfaces [6–10]. Sophisticated analytical techniques such as x-ray photoelectron spectroscopy allow for going into more detail and investigating chemical DNA modifications, e.g., dehydrogenation and oxygen loss [11]. However, the assignment of such reaction pathways to a particular damage mechanism remains ambiguous because of the interplay of direct and indirect damage.

The most straightforward approach to single out the direct effect is by use of molecular DNA building blocks in the gas phase. Gas-phase targets of these molecules are relatively easily produced by means of evaporation from an oven. Over the last decade, many studies have focused on molecular ionization and fragmentation dynamics upon interaction with energetic photons [12,13], low-energy electrons [14,15], and keV ions [16–20]. It was observed that among the small DNA building blocks deoxyribose molecules are particularly sensitive to irradiation. They disintegrate completely upon ionization by electrons [21], photons [13], and keV ions [22,23]. For keV ion impact, theoretical efforts have until now focused on determination of ionization and electron capture cross sections, e.g., in proton [24,25] and \( \text{C}^{+}+ \) [26,27] collisions with nucleobases.

With the wealth of existing data on the response of gas-phase DNA building blocks to interaction with energetic ions or photons, it is important to know how far these results can help in understanding the response of an entire strand of gas-phase DNA. Are gas-phase oligonucleotides most prone to dissociation at the sugar moieties whereas the nucleobases are more stable? Do known breakup channels of isolated building blocks show up in the fragmentation pattern of the much larger macromolecule?

A first hint came from experiments on keV-ion-induced fragmentation of nucleobases or amino acids in a cluster. In the cluster environment, new fragmentation channels open up that are closed for isolated molecules [28,29]. The structure and composition of homomolecular clusters differ strongly from those of DNA strands. Intermediate molecular systems such as nucleotides and oligonucleotides (see Fig. 1) are more appropriate to use as test beds bridging the gap between DNA and its smallest molecular building blocks. However, nucleotides and oligonucleotides decompose thermally already upon very moderate heating. To bring these species into the gas phase, it is necessary to employ electrospray ionization, laser desorption, or similar advanced techniques. The target densities that can be achieved by these techniques are usually much lower than those obtained by evaporation of, for example, the nucleobases. To circumvent this target-density problem it is possible to take the opposite route and study energetic collisions of electrosprayed oligonucleotide anions with a gas target of neutral noble gas atoms [30]. Collision experiments of this type have for instance shown that nanosolvation of the nucleotide adenosine 5-monophosphate can efficiently quench collision-induced fragmentation of the embedded molecule [31]. A limitation of this type of collision studies is that only relatively low collision energies can be reached in the center-of-mass system and therefore only very...
moderate amounts of excitation energy can be deposited in the biomolecule.

We have therefore chosen to investigate the impact of keV Cq+ ions and energetic photons on protonated tetranucleotides with sequences dGCAT (for a sketch of the structure, see Fig. 1) and dGTAT, stored in a radio-frequency trap. In the following we will present the resulting fragmentation patterns. The yields of the various dissociation products recorded as a function of photon energy and ion charge state q will be interpreted with emphasis laid on the role of DNA building blocks in comparison to their dissociation dynamics as free molecules.

II. EXPERIMENT

The photoionization experiments presented in this work have been carried out at the synchrotron beamlines MAXlab i411 (Lund, Sweden [32]) and BESSY II U125/2-NIM (Berlin, Germany [33]). The ion-induced fragmentation experiments were performed at the Zernike-LEIF facility at the KVI (University of Groningen, The Netherlands).

The experimental setup is displayed in Fig. 2 and has been described in detail before [34]. Briefly, an in-house-built electrospray ionization (ESI) source was used to spray a 40 μM solution of the oligonucleotides dGCAT and dGTAT (DNA technology, Risskov, Denmark) solvated in a mixture of 80% methanol, 20% water, and with 0.5% formic acid under atmospheric conditions. The ESI beam was then expanded into a first vacuum chamber housing a rf ion funnel (based on the design used by Julian et al. [35]) for phase space compression. After further phase space compression in a quadrupole rf ion guide, the cations entered a rf quadrupole mass filter, where selection of the doubly protonated oligonucleotides [dGCAT + 2H]2+ or [dGTAT + 2H]2+ took place. These dications were then transferred into a three-dimensional (3D) rf ion trap (base pressure $p \approx 1 \times 10^{-8}$ mbar) and accumulated to reach sufficient target density. For collisional cooling of the protonated oligonucleotides, a He buffer-gas pulse was applied. At typical operating conditions, the cooled target had a 300 μm diameter and contained a few thousand protonated oligonucleotides. The typical target number density was thus of the order of $10^8$ cm$^{-3}$.

To determine the mass distribution of the rf-trap content, the cations were then extracted into a linear time-of-flight (TOF) mass spectrometer ($M/\Delta M \approx 200$) by applying bias voltages of about ±200 V to the rf-trap end caps for 5 μs.

The rf trap was typically filled for about 1 s. A time interval of 100 ms allows for the buffer-gas pressure to decrease before exposure to ions or photons is started. Depending on the photon...
or ion flux, exposure times between 150 and 2000 ns were typically chosen such that less than 10% of the trap content was ionized. In this way it was ensured that not more than 10% of the trapped protonated oligonucleotides underwent multiple interaction processes. Subsequently, an additional short buffer-gas pulse (50 ms) was applied, to cool down energetic fragmentation products.

To compensate for contributions of residual gas, from each acquired spectrum, a subsequent second mass scan obtained under identical conditions but with an empty trap was subtracted. As a reference for the trap content, eventually a mass scan of the native trap content without ion or photon exposure, respectively, is also subtracted. Typically, this sequence of three mass scans is repeated 500–1000 times, to accumulate sufficient statistics.

For photon energies between 10 and 30 eV, the setup was interfaced with the BESSY II U125/2 beamline: Photons were generated in a quasiperiodic undulator consisting of 32 dipole magnet periods each 125 mm long, in combination with a 10-m-focal-length normal incidence monochromator [33]. For maximum photon flux of $\approx 10^{13}$ photons/s, a relatively low-resolution 300 lines/mm grating was employed. For the soft-x-ray photoabsorption studies, we used the MAXlabinstrument i411 beamline. Here, the undulator consisted of 43 dipole magnets of 59 mm length. A modified SX-700 monochromator equipped with a 1220 lines/mm grating delivered a flux typically exceeding $10^{12}$ photons/s. The photon flux was determined using a radiation hard silicon photodiode (model SXUV, IRD, Newbury Park, CA) and the exposure time was controlled using a mechanical shutter with a 14 mm aperture (Uniblitz, Rochester, NY). For the ion-induced fragmentation studies, the setup was interfaced to the Zernike-LEIF keV ion beamline. The ion current was measured using a Faraday cup with typical currents in the nA regime. Exposure time was controlled by means of an electrostatic deflection field in the keV ion beamline.

For keV ions, the rf-trap potentials strongly affect the ion-beam profile within the trap. Part of the broadened beam may miss the trapped molecules. To compensate for reduced beam overlap we normalized the mass spectra obtained at different ion energies to the loss of $[dGCAT+2H]^2+ \ cations$ from the trap. Throughout this article, for the cases of keV ion collisions, fragment ion yields are thus always relative with respect to the total ionization cross section. For photons the beam profile varies only with photon energy but stays an order of magnitude smaller than the diameter of the target volume. The loss of parent ions from the trap together with the absolute photon flux can thus be used to determine relative total photoabsorption cross sections as function of photon energy.

III. RESULTS

In the following, mass spectra and fragment ion yields from $[dGCAT+2H]^2+$ interactions with keV ions or energetic photons will be presented.

A. C$^+$ ions at keV energies

Figure 3 displays a mass spectrum of the cationic products obtained after collisions of 40 keV C$^+$ with $[dGCAT+2H]^2+$ (keV-ion-induced dissociation, KID). The latter has a monoisotopic mass of 1175.2 amu, i.e., the parent ion is found at $m/z = 587.6$ amu. A clear peak due to $[dGCAT+2H]^2+$ is evidence for non dissociative electron capture from the oligonucleotide dication. However, the spectrum is dominated by intense peaks in the low-mass range from $m/z = 60$ to $m/z = 160$ (note that ions with masses below 60 were not trapped). The strongest peak at $m/z = 81$ is likely to be mainly due to a $C_3H_5O^+$ fragment from the sugar moiety in the dGCAT backbone (see the discussion below). The next four peaks are actually doublets due to protonated nucleobase cations $[C+H]^+$ (cytosine, $m/z = 112$), $[T+H]^+$ (thymine, $m/z = 127$), $[A+H]^+$ (adenine, $m/z = 136$), and $[G+H]^+$ (guanine, $m/z = 152$) and their nonprotonated counterparts $T^+$, $A^+$, and $G^+$, with $m/z = 126$, 135, and 151. $C^+$ ($m/z = 111$) has negligible intensity. It is important to note, that the nomenclature can be ambiguous: The nucleobase fragments are formed by glycosidic bond cleavage, and therefore lack an A atom in comparison with their gas-phase counterparts. What we call the nonprotonated nucleobase cation $B^+$ and the protonated cation $[B+H]^+$ thus require addition of H and 2H, respectively.

Three peaks at $m/z = 192, 216$, and 232 have been previously observed in collision-induced dissociation (CID) studies [36] and were assigned to cyclic nucleoside complexes containing the five-membered sugar ring and the nucleobases cytosine, adenine, and guanine, respectively (see the inset in Fig. 3). A few other larger fragments observed with very small relative intensities will be discussed in the photoionization context. The relative yields of all fragments are summarized in Table I.

Zooms in the most relevant mass region $m/q = 60–160$ are displayed in Fig. 4 for 40 keV C$^+$ impact on $[dGCAT+2H]^2+$ and $q = 2–5$. For all $q$ the splitting of the nucleobase-related peaks into a protonated and an nonprotonated component is clearly visible for G, A, and T. For C, A, and G, the protonated cation is clearly stronger than the nonprotonated one. For T, the opposite is observed.
TABLE I. [dGCAT + 2H]2+ fragments formed upon 40 keV C4+ ion impact and photoabsorption (photons energies given in eV). Relative intensities are given in percent of the loss of parent ions from the trap. All fragments are singly charged cations unless stated otherwise. Note that m/z = 81 and 82 have also been observed with relatively small yields as nucleobase fragments formed in VUV photofragmentation [12]. For m/z = 83, 95, 97, 108, 109, and 110, only one of the possible assignments is given.

<table>
<thead>
<tr>
<th>Mass-to-charge ratio</th>
<th>Assignment</th>
<th>C4+</th>
<th>C4+</th>
<th>C+</th>
<th>C2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxyribose related</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>C4H5O</td>
<td>6.8</td>
<td>12.7</td>
<td>8.1</td>
<td>22.8</td>
</tr>
<tr>
<td>81</td>
<td>C4H5O</td>
<td>61.5</td>
<td>54.6</td>
<td>37.4</td>
<td>176</td>
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<td>82</td>
<td>C4H5O</td>
<td>23.2</td>
<td>5.9</td>
<td>3.3</td>
<td>22.6</td>
</tr>
<tr>
<td>99</td>
<td>C4H5O</td>
<td>6.1</td>
<td>9.4</td>
<td>5.1</td>
<td>22.2</td>
</tr>
<tr>
<td>Nucleobase related</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>C+ + H</td>
<td>55.3</td>
<td>33.8</td>
<td>19.1</td>
<td>55.8</td>
</tr>
<tr>
<td>126</td>
<td>T</td>
<td>1.9</td>
<td>5.7</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>127</td>
<td>T + H</td>
<td>0.3</td>
<td>4.1</td>
<td>0.1</td>
<td>1.7</td>
</tr>
<tr>
<td>135</td>
<td>A</td>
<td>2.6</td>
<td>4.1</td>
<td>3.4</td>
<td>3.8</td>
</tr>
<tr>
<td>136</td>
<td>A + H</td>
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<td>10.4</td>
<td>5.5</td>
<td>8.3</td>
</tr>
<tr>
<td>151</td>
<td>G</td>
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<td>7.5</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>152</td>
<td>G + H</td>
<td>7.8</td>
<td>13.7</td>
<td>8.4</td>
<td>7.6</td>
</tr>
<tr>
<td>83</td>
<td>C5H3NO, hν → T [12], H+ → C [37]</td>
<td>0.5</td>
<td>3.5</td>
<td>3.9</td>
<td>5.0</td>
</tr>
<tr>
<td>95</td>
<td>C5H3NO, hν → T [12], H+ → C [37]</td>
<td>1.5</td>
<td>1.3</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>97</td>
<td>C5H3NO, hν → T [12], H+ → C [37]</td>
<td>0.3</td>
<td>0.6</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>108</td>
<td>C5H3NO, hν → T [12], H+ → C [37]</td>
<td>0.8</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>109</td>
<td>C5H3NO, hν → T [12], H+ → C [37]</td>
<td>0.5</td>
<td>1.7</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>110</td>
<td>C5H3NO, hν → T [12], H+ → C [37]</td>
<td>1.0</td>
<td>1.7</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Nucleoside related</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>192</td>
<td>C + sugar moiety</td>
<td>0.5</td>
<td>0.8</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>216</td>
<td>A + sugar moiety</td>
<td>0.6</td>
<td>0.9</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>232</td>
<td>G + sugar moiety</td>
<td>0.3</td>
<td>0.7</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>392</td>
<td>[dGCAT + 2H]2+</td>
<td>2.6</td>
<td>3.2</td>
<td>4.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The relative intensities of the peak doublets vary strongly with q. The T peaks are always weakest and have appreciable intensity only for q = 4, whereas C is strongest for q = 2–5. For q = 2,3,4 we have also investigated collisions with [dGTAT + 2H]2+ (data not shown here). Interestingly, in that case the T peak becomes comparable in intensity to the G and A doublets but still almost no [T + H]+ is formed. The m/z 81 peak becomes relatively more pronounced.

For C4+ projectile ions, Fig. 5 displays the integrated relative yields for the nucleobase fragments as a function of projectile velocity v in atomic units (a.u.). All fragment yields exhibit an obvious decrease with v, which reflects the well known fact that for keV ions impinging on DNA building blocks, molecular excitation due to electronic stopping increases with the velocity [22,40]. The larger amount of excitation energy deposited in the molecule then leads to more extensive fragmentation and thus to a shift of the fragmentation pattern towards smaller masses. Because of the low-mass cutoff of the 3D ion trap, this is manifest in the mass spectra as a relative increase of the m/z 81 peak. Interestingly, the different nucleobase yields decrease differently with v.

Figure 6 therefore displays the ratio between protonated and nonprotonated cations of the nucleobases T, A, and G as a function of ion velocity v. It is clear that for T, the degree of protonation is always lower than 1 and systematically decreases with v. For A, very high degrees of protonation are observed which depend only weakly on v. A strong v dependence is observed for G, where the relative yields increase from 1.6 at v ≈ 0.28 a.u. to 2.3 at v ≈ 0.45 a.u.

Finally, Fig. 7 shows the ratios between protonated and nonprotonated nucleobase cations for constant v as a function of projectile charge state q. For q = 2–5, the ratios for T are consistently below 1 whereas for A and G, ratios around 2 are observed. For a given nucleobase, there is no apparent trend in the q dependence.

B. Energetic photons

For the complementary photoionization studies, mass spectra were recorded at several photon energies ranging from 10 up to 570 eV covering the entire range from valence photoionization to C, N, and O K-shell ionization.

Relative total photoabsorption cross sections as a function of photon energy can be directly obtained from the experimental data and are displayed in Fig. 8. The maximum due to molecular valence electrons is observed between 15 and 20 eV, in line with the findings for smaller biomolecules such as nucleobases [12]. The cross section then decreases with increasing photon energy until the K edges of C, N, and O are reached and the associated increase in cross section can be observed. To our knowledge, accurate 1s electron binding energies for gas-phase oligonucleotides, nucleotides, and even nucleosides are unknown. For nucleobase values of
FRAGMENTATION OF PROTONATED OLIGONUCLEOTIDES

FIG. 4. (Color online) Mass spectra of interaction products from 40 keV C\(^{q+}\) (\(q = 2–5\)) collisions with [dGCAT + 2H]\(^{2+}\).

290.7–294.8 eV (C), 404.2–407.1 eV (N), and 536.5–537.5 eV (O) are predicted by theory [41].

Note that cross sections and mass spectra have always been recorded for photon energies clearly above the K edge (330, 415, 430, and 570 eV) ensuring 1s ionization which is usually followed by an Auger deexcitation. Thus, two electrons are removed from [dGCAT + 2H]\(^{2+}\). Only for the C 1s shell have additional spectra been recorded just below the K edge (280 eV), in the 1s excitation regime (288.25 and 290 eV) and just above the K edge (300 eV).

FIG. 5. (Color online) Relative yields for protonated and nonprotonated nucleobases and for the m/z 81 fragment for collisions of C\(^{4+}\) with [dGCAT + 2H]\(^{2+}\) as a function of ion velocity v. The error bars would not exceed the symbol sizes.

A [dGCAT + 2H]\(^{2+}\) photofragmentation spectrum obtained at 40 eV photon energy is shown in Fig. 9 and a list of the most common fragments can be found in Table I. At this photon energy, essentially the same fragments are observed that are formed upon keV ion impact (see Fig. 4). The strongest peaks are again due to intact protonated and nonprotonated nucleobases and the m/z = 81 fragment, which is most likely due to C\(_5\)H\(_5\)O\(^+\) from the deoxyribose moiety. Also the nucleoside complexes with m/z = 192, 216, and 232 (containing C, A, and G, respectively) can be clearly recognized (see the inset in Fig. 4). A number of weaker peaks in Fig. 9 are found at m/z = 69 (tentatively assigned to C\(_2\)H\(_2\)O\(^+\)), 178 (sugar moiety + phosphate group, C\(_5\)H\(_5\)O\(_5\)P\(^+\)), 219 (not assigned), 392 ([M + 2H]\(^{3+}\)), 412 ([p + dA + p]\(^+\), where p is the phosphate group moiety and dA stands for deoxyadenosine), 427 ([a\(_2\) − C]\(^+\) or a\(_3\)\(^+\)), and 463 (w\(_2\)\(^+\)). Two larger fragments with relatively small yields are found at m/z = 636 (not assigned) and 700 ([a\(_3\) − G]\(^+\)) (not shown in Fig. 9). (Here, we follow the usual notation with a\(_i\) and w\(_j\) being ions containing the 5’ and 3’ terminus, respectively,
formed by phosphodiester bond scission at the deoxyribose 5′ carbon.)

Figure 10 compares [dGCAT + 2H]^{2+} photofragmentation spectra obtained at photon energies of 15, 40, 330, 430, and 570 eV. In all spectra essentially the same fragment cations are formed. However, the relative yields depend strongly on photon energy. At 15 eV the spectrum is dominated by the G and A doublets. C is a bit weaker and the m/z = 81 fragment is very weak. The [dGCAT + 2H]^{2+} peak due to nondissociative photoionization is more prominent than for Cq^{+} ion impact. An increase in photon energy to 40 eV leads to strong changes in the fragmentation pattern, which shifts to smaller masses. The m/z = 81 peak becomes strong, and nondissociative ionization is reduced as are the intensities of the larger fragments. The nucleobase peak doublets are of comparable intensity to the m/z = 81 peak. In contrast to the 15 eV case, at 40 eV C is strongest and T becomes visible. At 330, 430, and 570 eV, i.e., beyond the C, N, and O K edges, respectively, very similar fragmentation patterns are observed. Here, nondissociative ionization is no longer observed and larger fragments no longer appear. The m/z = 81 peak dominates over the nucleobase doublets, which are clearly visible, again with C being strongest.

Figure 11 displays the photofragmentation yields for C, T, A, and G in their protonated and nonprotonated forms as a function of photon energy. No sizable yield of nonprotonated C (m/z = 111) is observed over the whole range. For all fragments, the yields show a similar trend to that of the total photoabsorption cross section (Fig. 8) and the yields of the protonated species always exceed the respective nonprotonated ones. However, clearly different slopes are observed when comparing protonated and nonprotonated fragment cations. This is visualized in Fig. 12 where the ratio of protonated to nonprotonated species as a function of photon energy is displayed.

### IV. DISCUSSION

Nucleobases dominate the fragmentation patterns for both ions and photons. The following sections will therefore focus on the determination of the [dGCAT + 2H]^{2+} initial protonation sites. Subsequently, the yields of protonated and nonprotonated nucleobase cations will be discussed in the contexts of photoionization and electron capture. The variation of the ratios between protonated and nonprotonated nucleobase cations with photon energy, ion velocity v, and ion charge state q will be examined with emphasis laid on the excitation energies and time scales. Eventually, DNA damage
A. Pathways to nucleobase fragment ions: The initial protonation site

For keV ion collisions as well as for absorption of energetic photons, the dominating dissociation products are protonated and nonprotonated nucleobase cations C, T, A, and G. In early low-energy CID studies on various gas-phase protonated dinucleotides, Phillips and McCloskey [42] have investigated the formation of protonated nucleobase cations. The underlying fundamental process was found to be cleavage of the glycosidic bond between a sugar and a protonated nucleobase followed by a hydrogen transfer from the sugar to the base. Assuming a similar scenario for KID and photofragmentation, the most probable protonation sites of \([dGCAT + 2H]^2+\) need to be known to quantitatively understand the relative intensities of the nucleobase-related cation yields. Neutral oligonucleotides in solution are believed to exist in a zwitterionic form, with the negative charge on the phosphate group being neutralized by positively charged nucleobases. For dGCAT, the approximate distribution of protonation sites over the nucleobases can be estimated using the \(pK_a\) values of the different nucleosides. The 0.5% solution of formic acid used for the ESI has a \(pH\) of about 2.3. With the \(pK_a\) values from Table II it follows that the C, A, G, and T moieties are protonated to about 100%, 90%, 75%, and 0%, respectively.

This protonation site ratio can be assumed to be preserved upon transfer into the gas-phase [50]. This is due to the higher proton affinities (PAs) of the four nucleobases as compared to the sugar or phosphate groups within the oligonucleotide. Table II lists gas-phase PAs of different DNA building blocks. The PAs for nucleobases are within a narrow range of \(\approx 225–230\) kcal mol\(^{-1}\) for A, C, and G, while T has

<table>
<thead>
<tr>
<th>Molecule</th>
<th>(PA) (kcal/mol)</th>
<th>(pK_a)</th>
<th>Vertical IE (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA bases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine G</td>
<td>229.3 [44]</td>
<td>8.26 [45]</td>
<td></td>
</tr>
<tr>
<td>Cytosine C</td>
<td>227.0 [44]</td>
<td>8.89 [46]</td>
<td></td>
</tr>
<tr>
<td>Adenine A</td>
<td>225.3 [44]</td>
<td>8.47 [46]</td>
<td></td>
</tr>
<tr>
<td>Nucleosides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dG</td>
<td>237.9 [44]</td>
<td>2.9–3.3 [47,48]</td>
<td></td>
</tr>
<tr>
<td>dC</td>
<td>236.2 [44]</td>
<td>4.3–4.4 [47,48]</td>
<td></td>
</tr>
<tr>
<td>dA</td>
<td>237.0 [44]</td>
<td>3.8–4.1 [47,48]</td>
<td></td>
</tr>
<tr>
<td>dT</td>
<td>226.7 [44]</td>
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<td></td>
</tr>
<tr>
<td>Deoxyribose (furanose)</td>
<td>9.9 [47,48]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate group</td>
<td>220–225 [43]</td>
<td>10.06 [49] (t)</td>
<td></td>
</tr>
</tbody>
</table>
a substantially lower value of $\approx 210$ kcal mol$^{-1}$. For the corresponding nucleosides, the PAs are systematically larger but follow the same trend as for the nucleobases. The higher PA of nucleosides as compared to the free bases is due to intramolecular hydrogen bonding between the sugar and base and the electron-donating action of the sugar towards the phosphate moiety. The PA value for the phosphate group is expected to lie in the range of 220–225 kcal mol$^{-1}$ and clearly falls short of the nucleoside PAs.

Therefore, in the target [dGCAT + 2H]$^{2+}$ ions, C is expected to be 100% protonated whereas T is 100% unprotonated. The second proton is expected either on A or G with a preference for A. Exactly this order is found for photofragmentation over the whole range of wavelengths under study (see Table I). In particular the low T yields are in line with CID studies: Vrkic et al. (see Table I and Fig. 5). In particular the low T yields are in line with CID studies: Vrkic et al. (see Table I and Fig. 5). In particular the low T yields are in line with CID studies: Vrkic et al. (see Table I and Fig. 5).

Ion impact or photoabsorption triggers the cleavage of the C-N glycosidic bond of a protonated site, leading to formation of the nonprotonated nucleobase cations. In a slower process, the resulting N radical (homolytic cleavage) abstracts a H radical or a proton, respectively, from the sugar moiety, and a protonated nucleobase cation is formed. Clearly, this second pathway is possible only if the initial cleavage process is sufficiently slow. Since T is almost certainly not protonated in [dGCAT + 2H]$^{2+}$, the formation of T$^+$ (m/z 126) and TH$^+$ (m/z 127) by either KID or photoionization must have a very different precursor than for the formation of G$^+$, GH$^+$, CH$^+$, A$^+$, and AH$^+$. The corresponding processes are sketched in Fig. 14(a).
For T, a radical hole is localized on the sugar moiety, leading to rapid β-bond cleavage and release of a neutral (and thus undetected) thymine base ($m/z$ 125), and a [dGCA + 2H$^+$]-sugar radical cation, which can be formed by O-C5' bond scission from the sugar. The remaining sugar radical cation can dissociate, leading to the observed fragments $m/z$ 69, 81, and 99. The remaining [dGCA + 2H$^+$] ion ($m/z$ 475) is observed as a weak peak in low-energy photoionization (see Fig. 9) and in traces in KID. It can, however, be assumed that further fragmentation is a more likely process. The preferential hole localization on the sugar moiety has recently been directly observed in photoionization studies of neutral gas-phase thymidine.[52]: Whereas C is photoionization leads only to formation of small fragments, 50 eV photons induced formation of approximately equal yields of T$^+$ and TH$^+$, which in total amount to only half the yield of the sugar moiety fragment.

Alternatively, the initial radical hole produced on the thymine sugar moiety leads to rapid proton transfer from C1' or C2' to the T moiety [Fig. 14(b)], followed by rapid β-bond cleavage [Fig. 14(c)], i.e., formation of a T$^+$ radical cation ($m/z$ 126). We do not observe the remaining [dGCA + 2H$^+$]-sugar moiety ($m/z$ 524.6), i.e., it can be assumed that it fragments further to yield other (observed) fragments.

However if after step (b), β-bond cleavage is slow enough, a neutral H can be transferred, from, e.g., C2' to the thymine moiety during β-bond cleavage, leading to [T + H]$^+$ ($m/z$ 127), i.e., a protonated free base [Fig. 14(d)]. Again, nonobservation of the [dGCA + 2H$^+$]-sugar moiety suggests further fragmentation into other ion fragments.

**B. Pathways to nucleobase fragment ions: Protonated vs nonprotonated species**

Nonprotonated nucleobase cations as observed in our experiments are usually not seen in CID spectra of protonated oligonucleotides. Their occurrence in KID and photofragmentation thus is most likely related to the ion- or photon-induced [dGCAT + 2H$^+$] ionization and is not necessarily a reflection of the initial protonation site. Clearly, nonprotonated fragments can be formed along the two-step pathway sketched above, when the glycosidic bond scission occurs at an unprotonated site, or they can be formed from a protonated site, when the H abstraction from the sugar is hindered. As explained before, in [dGCAT + 2H$^+$], C is always protonated and T is always unprotonated. One of the purines A and G then is protonated, and the other one unprotonated. The ratio of protonated to nonprotonated ions in the mass spectra will then be determined by the ability (1) to break the C-N bond of either a protonated or an unprotonated base and (2) the ability of the resulting radical (or ion) to abstract a H radical or a proton from the sugar moiety.

Assuming photoabsorption on a random site of the molecule, C-N cleavage is thermodynamically most likely for pathways leading to the most stable products. The protonated radical cation formed from a protonated base (see Fig. 13) is more stable than a neutral radical formed from an unprotonated species and C-N cleavage (1) should be most likely for protonated bases. The fact that for the 100% protonated C, only protonated fragment cations are observed for KID and photofragmentation suggests that H radical abstraction (2) is a very likely process. Additionally, in the less likely case of C-N cleavage for an unprotonated base, H$^+$ abstraction leads to a nonprotonated fragment cation whereas abstraction of a neutral H or no H at all leaves the fragment in a neutral and thus undetectable state. For the purine bases, cleavage of the C-N bond involving the protonated base is more likely and will most probably lead to formation of a protonated base. Cleavage of the C-N bond for the unprotonated base is less likely and the product might stay an undetected neutral.

This explains the high GH$^+$:G$^+$ and AH$^+$:A$^+$ ratios observed in KID (see Figs. 6 and 7) and photofragmentation below the $K$ edges (see Fig. 12). For the 100% unprotonated...
T, protonated base cations can be formed only after proton migration. For KID, TH⁺:T⁺ ratios between 0.5 and 0.7 are observed (see Figs. 6 and 7). For photoionization this ratio is larger than 1.

How does the ionization process itself come into play? In pioneering KID studies on polypeptides [34,53] it was shown that glancing-ion trajectories contribute most to the mass spectra. This is because head-on collisions lead to excitation energies in the range of tens of eV, inducing extensive multifragmentation into small fragments with masses below the trapping limit employed here. The dominant mechanism in glancing ion-molecule collisions is resonant valence-electron capture. KID involving single-electron capture is thus expected to trigger fragmentation mechanisms very similar to those following valence photoionization by VUV photons. In our experiments, this is for instance reflected in the observation of sizable branching ratios for nondissociative ionization in KID (for instance, formation of 4.2% [dGCAT + 2H]⁺ after C⁺ collisions; see Table I). For the case of photoionization, a similarly high [dGCAT + 2H]⁺ yield of 9.9% is observed for a photon energy of 15 eV which allows only for photoionization of the highest occupied molecular orbitals. Nondissociative ionization decreases dramatically with increasing photon energy.

A crucial quantity in the context of ionization is the vertical ionization energy (IE). In nucleotides and DNA the lowest local vertical IEs are found on the nucleobase moieties. As is obvious from Table II, the highest vertical IE of an isolated nucleobase is found for T, whereas the purine nucleobases A and in particular G are lowest [45,46]. This ordering is unaffected by the presence of deoxyribose and phosphate groups and reflects the well-known fact that holes in DNA are usually trapped at the purine bases [54]. This ordering is fairly well reproduced in the valence photoionization data (photon energies below the C K edge) and to a much weaker extent in the KID data (see Table I): G⁺ is always strongest and T⁺ is almost always weakest. Induced holes apparently migrate through the oligonucleotide and get trapped at the nonprotonated base which has the lowest IE. Accordingly, C⁺ is not observed at all, because it is 100% protonated.

For the projectile ion charge states q = 2–5 under investigation here, double (or multiple) electron capture will contribute as well. In this case, KID is expected to resemble deeper photoionization processes. Above-threshold K-shell photoionization is usually followed by an Auger-type deexcitation process, leading to double ionization of the [dGCAT + 2H]²⁺ parent molecule accompanied by substantial molecular excitation. High photon energies which are still below the C K edge can induce a variety of other processes, most of which involve deposition of high excitation energies with the possibility of loss of additional electrons. Qualitatively, this is in line with the observation that fragmentation patterns in the low-mass range are very similar for C⁺ impact on [dGCAT + 2H]²⁺ and for photoionization with higher photon energies. Multiple ionization and/or higher excitation are also reflected in the yields of nonprotonated nucleobase cations. As mentioned above, for KID where single- and multiple-electron removal contribute, the prevalence of G⁺ is relatively weak and A⁺ and T⁺ have comparable yields for all q. For photoionization above the C K edge, A⁺ becomes comparable to G⁺ but T⁺ remains low (see Table I).

C. Protonated vs ionized nucleobases: Excitation energy and interaction time

In contrast to CID, photoionization and KID are processes in which charge is removed (and energy is deposited) in a single collision event. Given the size of the oligonucleotides under study, even multiple-electron transfer to a C⁺ projectile ion can be considered a localized process. Furthermore, the time scales of collision or photoionization processes are ultrashort in comparison to typical time scales for internal vibrational redistribution of excitation energy. Most likely this leads to modified fragmentation mechanisms. It is difficult to recognize the consequences of such ultrafast processes from the fragment yields alone. The mere amount of deposited energy strongly influences these yields, as is obvious from Figs. 5 and 11, where for instance all yields of nucleobase-related fragments decrease with increasing C⁺ velocity v or (over a wide range) with photon energy. Ratios between fragment yields stemming from competing channels, however, can be much more sensitive. The ratio between protonated and nonprotonated nucleobase cations shown in Fig. 6 clearly shows a relative increase of [G + H]⁺ as compared to G⁺. Clearly, an increase of v (leading to shorter interaction times and higher excitation energies) hinders hole migration towards G. For T, the opposite trend is observed. Here, hole migration away from T becomes hindered. The fact that for T a ratio smaller than 1 is found whereas for A and G, the ratios are of the order of 2 is readily explained by the previously mentioned low PA of T, which is unlikely to be protonated in the first place.

For photoionization a completely different dependence of the ratios on the photon energy is observed (see Fig. 12): Below the C K edge, for T the protonated species is found to increase in comparison to the nonprotonated one, whereas for G a moderate decrease is observed. For A, a dramatic decrease is even found. Only for G are the ratios quantitatively similar to those in KID. It could be assumed that with increasing photon energy, even below the C K edge, multiple ionization starts to play a role. In KID, the projectile-ion charge state q has an influence on electron removal. However, Fig. 7 shows that the effect of increasing q from 2 to 5 on the protonation ratios is only weak.

D. Backbone scission and the fragmentation of DNA building blocks

The H-abstraction step leading to formation of a protonated or a nonprotonated nucleobase fragment (see Fig. 13) is likely to trigger the formation of a sugar-moiety-based dehydrated fragment with m/z = 81 (C₆H₁₀O), which involves backbone scission [42]. This process is indicated as a last step in Fig. 13. It is possible that this fragment transforms into a six-membered ring. An alternative assignement of m/z = 81 to PO₃H₂ derived from the phosphate group was ruled out by deuterium exchange [42]. For KID m/z = 81 dominates the spectra for all charge states and ion kinetic energies. In the photofragmentation case, m/z = 81 is very weak for photon
energies around 10 eV. However, it becomes comparable to the stronger nucleobase peaks already at 40 eV. Beyond the $K$ edges of C, N, and O, similarly to KID, this fragment dominates (see Fig. 11 bottom). The related fragments with $m/z = 69$ and 82 are much weaker in intensity. The peak at $m/z = 99$ can be assigned to a deoxyribose fragment also related to $m/z = 81$ but stemming from either the 5- or the 3-terminal deoxyribose moiety.

It is interesting to note that in collisions with keV ions [22] (and even 70 eV electrons [21]) with gas-phase deoxyribose, C$_5$H$_5$O is observed only with very small relative yields. VUV photoabsorption in gas-phase deoxyribose does not lead to formation of this fragment either [13] and also in soft-x-ray-induced damage to deoxyribose thin films, the yield of $m/z = 81$ fragments is negligible [55]. Why is the fragmentation of gas-phase oligonucleotides so different from its building blocks? First of all, it is not a priori clear that gas-phase deoxyribose has the same five-membered ring structure (furanose form) of its DNA counterpart. A different molecular structure can obviously lead to different fragmentation channels [36]. Second, the fragments observed do not necessarily stem from the ion-impact or photoabsorption site in the macromolecule. In the case of localized damage, the remaining oligonucleotide might dissociate in a way totally unrelated to that of the impact site. Second, the chemical environment is known to dramatically change fragmentation. Last but not least, the excitation energy might be distributed over the entire molecule before dissociation sets in, efficiently “cooling down” the impact or photoabsorption site. To summarize this, the [GCAT + H]$^{+}$ fragmentation data clearly confirm that the deoxyribose moiety plays an important role in radiation damage of DNA, as was concluded in most gas-phase experiments on isolated deoxyribose molecules but only fragments unobserved in the gas phase occur.

Besides the gas-phase deoxyribose data, various gas-phase studies have been performed on nucleobases. In the gas phase nucleobases prove to be more stable than deoxyribose, which is clearly reflected in the [GCAT + H]$^{+}$ fragmentation—intact nucleobase cations always dominate the mass spectra. However, in contrast to the case of deoxyribose, a number of nucleobase fragments are observed, which is found for the gas-phase species as well (see Table I). In VUV photofragmentation, the peaks at $m/z = 83$ and 97 were for instance found for T [12], $m/z = 95$ for C [38], $m/z = 108$ for A [12], and $m/z = 109$ and 110 for G [38]. For ion impact $m/z = 108$ was observed for A [39] and $m/z = 83$ for C [37]. Nucleobase fragmentation in oligonucleotides is thus a non-negligible but weak channel, which leads to fragments also observed for isolated gas-phase nucleobases.

Finally, if the backbone scission leading to the $m/z = 81$ fragment is not preceded by nucleobase loss, fragment cations with $m/z = 192, 216$, and 232 can be formed, which correspond to C$_4$H$_5$O plus the nucleobase cytosine, adenine, and guanine, respectively (see Fig. 9). No thymine-containing fragment is formed—most probably again due to the low thymine proton affinity already discussed. These nucleoside-related fragments are observed in all KID spectra. For photofragmentation, the corresponding peaks are strong only for lower photon energies and almost absent when the $K$ edges are reached. Clearly, multiple ionization and higher excitation energies quench these fragments since more extensive multifragmentation is induced.

An important finding of our study is that photofragmentation at the $K$ edge of either C, N, or O leads to virtually identical mass spectra. The nonspecificity of the photoabsorption site is not a priori expected because in DNA, N is exclusively found in the nucleobases (see Fig. 1). Nevertheless, this result is in agreement with studies on dry DNA in the condensed phase, where no characteristic effect was observed for photoionization above ($h\nu = 2147$ eV) and below ($h\nu = 2153$ eV) the phosphorus $K$ shell.

V. Conclusions

In this article we have investigated the response of isolated protonated oligonucleotides upon keV ion impact and upon absorption of energetic photons. Qualitatively similar fragmentation patterns were observed. The spectra were found to be dominated by a deoxyribose fragment with $m/z = 81$ and protonated and nonprotonated nucleobase cations. Deoxyribose fragments contribute strongly to the mass spectra but the fragment masses are typically unobserved in gas-phase studies. In agreement with the gas-phase studies, however, deoxyribose seems to be involved in most fragmentation channels. For nucleobases, oligonucleotide fragmentation qualitatively reflects the gas-phase data: Nucleobases appear to be very stable and all observed fragments have previously been described in gas-phase studies. However, our study proves that great care must be taken when directly comparing gas-phase data obtained for a DNA building block with results for more complex systems.

Nucleobase protonation largely reflects the energetic ordering of the nucleobase proton affinities. The ratio between formation of protonated and nonprotonated species depends strongly on ion velocity and photon energy. For ions the results indicate that more energetic collisions hinder proton migration towards guanine.

In the future we plan to perform similar experiments with larger oligonucleotides in their de-protonated form. Such species are expected to allow an even more realistic look at molecular mechanisms underlying biological radiation damage.

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HYDROGENATION OF PAH CATIONS: A FIRST STEP TOWARD H$_2$ FORMATION

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ABSTRACT

Molecular hydrogen is the most abundant molecule in the universe. A large fraction of H$_2$ forms by association of hydrogen atoms adsorbed on polycyclic aromatic hydrocarbons (PAHs), where formation rates depend crucially on the H sticking probability. We have experimentally studied PAH hydrogenation by exposing coronene cations, confined in a radio-frequency ion trap, to gas phase atomic hydrogen. A systematic increase of the number of H atoms adsorbed on the coronene with the time of exposure is observed. Odd coronene hydrogenation states dominate the mass spectrum up to 11 H atoms attached. This indicates the presence of a barrier preventing H attachment to these molecular systems. For the second and fourth hydrogenations, barrier heights of 72 ± 10 meV, respectively, are found, which are in good agreement with theoretical predictions for the hydrogenation of neutral PAHs. Our experiments, however, prove that the barrier does not vanish for higher hydrogenation states. These results imply that PAH cations, as their neutral counterparts, exist in highly hydrogenated forms in the interstellar medium. Due to this catalytic activity, PAH cations and neutrals seem to contribute similarly to the formation of H$_2$.

Key words: astrochemistry – ISM: molecules

Online-only material: color figures

1. INTRODUCTION

Molecular hydrogen is the most abundant molecule in the universe and the main constituent of regions where stars are forming. H$_2$ plays an important role in the chemistry of the interstellar medium (ISM), and its formation governs the transformation of atomic diffuse clouds into molecular clouds. Because of the inefficient gas phase routes to form H$_2$, dust grains have been recognized to be the favored habit to form H$_2$ molecules (Oort & van de Hulst 1946; Gould & Salpeter 1963). The sticking of H atoms onto surfaces has received considerable attention because this mechanism governs the formation of H$_2$, but also other molecules that contain H atoms. The sticking of H atoms onto dust grains can also be an important mechanism to cool interstellar gas (Spaans & Silk 2000). In the past few decades, a plethora of laboratory experiments and theoretical models have been developed to understand how H$_2$ forms. As H atoms arrive on dust surfaces, they can be weakly (physisorbed) or strongly (chemisorbed) bound to the surface. The sticking of H$_2$ has been highlighted by several experiments on different types of surfaces (amorphous carbon, silicones, and graphite).

In the ISM, dust grains are mainly carbonaceous or silicate particles with various sizes and represent an important surface for the formation of H$_2$. However, a large part (∼50%) of the available surface area for chemistry is in the form of very small grains or polycyclic aromatic hydrocarbons (PAHs; Weingartner & Draine 2001). These PAHs are predicted to have characteristics similar to graphite surfaces; however, once the first H atom is chemisorbed on the basal plane, subsequent adsorptions of H atoms in pairs appear to be barrierless for the para dimer and with a reduced barrier for the ortho dimer (Rougeau et al. 2006). H$_2$ can then form by involving a pre-adsorbed H atom in monomer (Sha & Jackson 2002; Morisset et al. 2003, 2004; Martinazzo & Tantardini 2006) or in a para-dimer configuration (Bachellerie et al. 2007). However, while these routes represent efficient paths to form H$_2$, the inefficient sticking of H atoms in monomers constitutes an important obstacle to enter the catalytic regime for H$_2$ formation. This results in a very low H$_2$ formation efficiency on graphitic/PAH surfaces (Cazaux et al. 2011).

The hydrogenation on the PAH edges has been identified as an important route to form H$_2$ in the ISM (Bauschlicher 1998; Hirama et al. 2004; Le Page et al. 2009; Mennella et al. 2012; Thrower et al. 2012). Density functional theory calculations have shown that the first hydrogenation of neutral coronene is associated with a barrier (∼60 meV) but that subsequent hydrogenation barriers vanish (Rauls & Horneck 2008). Recently, coronene films exposed to H/3D atoms at high temperature were studied by means of IR spectroscopy (Mennella et al. 2012) and mass spectrometry (Thrower et al. 2012). These measurements showed that neutral PAHs, when highly hydrogenated, are efficient catalysts for the formation of H$_2$, and confirmed the high H$_2$ formation rate attributed to PAHs in photodissociation regions (PDRs; Mennella et al. 2012).

PAH cations, which are usually present at lower extinction $A_V$, and therefore reside at the surfaces of PDRs, also represent an important route to form H$_2$ (Bauschlicher 1998; Le Page et al. 2009). The addition of the first H atom is predicted to be barrierless. This reaction is exothermic but the product should be stabilized by IR emission. A second H atom can react with the already adsorbed H to form H$_2$ without a barrier (Bauschlicher 1998; Hirama et al. 2004).

In this Letter, we study experimentally the hydrogenation of coronene cations in the gas phase through exposure to hydrogen atoms. By using mass spectrometry, we show that odd hydrogenation states of coronene cations predominantly populate the mass spectrum. Our results highlight the fact that the further hydrogenation of PAH cations is associated with a barrier if the number already attached H atoms is odd, and no
2. EXPERIMENTS

In this pilot experiment, we show the feasibility of studying the hydrogenation of PAHs in the gas phase. For this purpose, we use a setup designed to study molecular ions in a radio-frequency (RF) ion trap. Time-of-flight (TOF) mass spectrometry of the trap content is used to identify the changes in mass of the coronene cations and therefore deduce their respective degrees of hydrogenation.

2.1. Setup

The experiments have been performed using a home-built tandem-mass spectrometer shown schematically in Figure 1 (Bari et al. 2011). A beam of singly charged coronene radical cations ([C_{24}H_{12}]^+, m/z 300) was extracted from an electrospray ion source. The ions were phase-space compressed in an RF ion funnel and subsequently in an RF quadrupole ion guide. Mass selection was accomplished by using an RF quadrupole mass filter. Accumulation of the ions took place in a three-dimensional RF ion trap (Paul trap). An He buffer gas at room temperature was used to collisionally cool the trapped cations. Exposure to gas-phase atomic hydrogen for variable periods of time led to multiple hydrogen adsorption on the coronene cations. An electric extraction field was then applied between the trap end caps to extract the trapped hydrogenated cations into a TOF mass spectrometer with a resolution of M/ΔM ~ 200. To obtain mass spectra of sufficient statistics, typically a couple of hundred TOF traces were accumulated.

Electrospray ionization allows us to gently transfer ions from the liquid phase into the gas phase. Inspired by the method of Maziarz (2005), we have run the ion source with a solution consisting of 600 μL of saturated solution of coronene in methanol, 350 μL of HPLC grade methanol, and 50 μL of 10 mM solution of AgNO_3 solution in methanol. In the liquid phase, electron transfer from a coronene molecule to a silver ion leads to formation of the required radical cation.

The trapped ions are exposed to hydrogen atoms produced from H_2 by a Slevin type source which has been extensively used in crossed beam experiments (Hoekstra et al. 1991; Biek et al. 1997). While in the earlier work the dissociation fractions were determined by means of electron impact excitation or He II line emission, we now use charge removal (captured ionization) and dissociation induced by 40 keV He^2+. For these processes, the cross sections are well known (Shah & Gilbody 1978). In this way, we determine a hydrogen dissociation fraction of n(H)/(n(H)+n(H_2)) ≈ 0.3. The temperature of the H beam is around room temperature (~25 mK).

2.2. Results

Coronene ions are exposed to a constant flux of H atoms for different periods of time before their degree of hydrogenation is determined by means of mass spectrometry. The irradiation time is varied from 1.0 up to 30 s to study the time dependence of coronene hydrogenation.

The data obtained from our experiment are a series of mass spectra of hydrogenated coronene cations as a function of H exposure time. Some of the spectra are shown in Figure 2. Figure 2(a) shows the mass spectrum of the native m/z = 300 coronene cations. A similar, thus unchanged, mass spectrum is obtained (not shown in this article) if we irradiate coronene cations with molecular hydrogen. This means that molecular hydrogen does not stick to coronene cations at room temperature.

After turning on the hydrogen source and exposing the coronene cations to the atomic hydrogen beam for 1.0 s (Figure 2(b)), the peak at m/z = 300 shifts to 301, which means that the trap content main constituent is (C_{24}H_{12}+H)^+. For increasing irradiation time (Figure 2(c) t = 2 s, (d) 3 s, (e) 4 s, and (f) 4.75 s), the peak at m/z = 301 disappears progressively while a peak at m/z = 303 and then at m/z = 305 (for t = 4.75 s; see Figure 2(f)) appears, which indicates the addition of three and five hydrogen atoms, respectively. At longer exposure time (t ~ 15 s; Figure 3(a)), the m/z = 303 peak dominates the signal, and a peak at m/z = 305 appears. At even longer irradiation times (t ~ 30 s; Figure 3(b)), the peak m/z = 305 dominates and peaks at m/z = 307 and 309 appear. These peaks clearly show the evolution of the hydrogenation states of coronene cations with H irradiation time.

3. ANALYSIS AND DISCUSSION

Our results show that the most important peaks measured in the mass spectrum shift from lower masses to higher masses with increasing H exposure time. In order to follow the evolution of the first hydrogenated state of coronene cation (C_{24}H_{12}+H)^+ (CorH^+) to the second (C_{24}H_{12}+2H)^+ (CorH^+), and fourth (CorH^+) hydrogenated states, we use a simple model that describes this evolution:

\[ \frac{dn_{\text{CorH}^+}}{dt} = -A_2 e^{-\frac{E_{\text{CorH}^+}}{k_B T}} \frac{C_{\text{CorH}^+}}{n_{\text{CorH}^+}} n_{\text{H}}. \]  

\[ \frac{dn_{\text{CorH}^+}}{dt} = A_3 e^{-\frac{E_{\text{CorH}^+}}{k_B T}} n_{\text{CorH}^+} - A_4 e^{-\frac{E_{\text{CorH}^+}}{k_B T}} n_{\text{CorH}^+} n_{\text{H}}. \]  

\[ \frac{dn_{\text{CorH}^+}}{dt} = A_5 n_{\text{CorH}^+} - A_6 e^{-\frac{E_{\text{CorH}^+}}{k_B T}} n_{\text{CorH}^+} n_{\text{H}}. \]  

\[ \frac{dn_{\text{CorH}^+}}{dt} = A_7 n_{\text{CorH}^+} - A_8 n_{\text{CorH}^+} n_{\text{H}}. \]  

Hydrogenation of CorH_{n=2}^+ follows an Arrhenius expression where A_{2n+2} is the prefactor and E_{2n+2} is the barrier, while hydrogenation of CorH_{n=3}^+ follows the same expression with a prefactor A_{2n+3} and no barrier, k_B is the Boltzmann constant and T the temperature of the H beam (T ~ 25 mK).
In these equations, we do not include abstraction, meaning that the time evolution of the contribution of each state is governed entirely by hydrogenation. This assumption is made in order to derive the first barriers of hydrogenation. Abstraction can be neglected in the conditions of our experiments for low exposure times. This is supported by previous experiments where the cross section for the addition of hydrogen to neutral coronene is predicted to be 20 times that for abstraction (Mennella et al. 2012). Further support is drawn from a kinetic chemical model we developed, which shows that abstraction must be very low compared to hydrogenation to be able to mimic the experimental results (Boschman et al. in prep). However, for long H exposure time we expect the hydrogenation degree of the coronene cations to reach a steady state which will allow us to derive the contribution of abstraction relative to addition, and therefore derive the H₂ formation rate due to PAH cations. It should also be kept in mind that in the conditions of our experiments, the H atoms are at room temperature, meaning that they cross the barriers for abstraction (10 meV; Rauls & Hornekær 2008) and addition (40–60 meV; Rauls & Hornekær 2008) with similar ease. Under interstellar conditions, however, the abstraction will dominate by eight orders of magnitude (at 20 K) because of the barrier differences.

The first hydrogenation is expected to take place at the outer edge carbon atom (Hirama et al. 2004). This state provides more conformational freedom to the four neighboring outer edge carbon atoms, ensuring a preference for the second hydrogenation to take place at one of those four carbon atoms. The third hydrogenation will preferentially take place at the outer edge carbon next to the second H atom. Again, the fourth H atom can be bound to one of the four neighboring outer edge carbon atoms, and the fifth sticks on the neighboring outer edge carbon. This scenario of H atoms sticking preferentially on outer edge carbons next to already adsorbed atoms is described in Rauls & Hornekær (2008).

The contribution of every peak is determined by fitting our data with Gaussians with identical widths (see Figure 4(a)). The ratios between different hydrogenation states as a function of time are reported in Figure 4(b). It appears that the ratio between the contribution of the first (CorH⁺) and the
second (CorH\textsuperscript{+}) hydrogenation states do not evolve with time for short timescales ((n\textsubscript{CorH\textsuperscript{+}}/n\textsubscript{CorH\textsuperscript{2+}}) \sim 3\textsuperscript{+} until 5\textsuperscript{+}). Also, the ratio between the third (CorH\textsuperscript{3+}) and the forth (CorH\textsuperscript{4+}) hydrogenation states shows identical behavior after \( t \geq 2\textsuperscript{+} \) s ((n\textsubscript{CorH\textsuperscript{3+}}/n\textsubscript{CorH\textsuperscript{4+}}) \sim 3\textsuperscript{+} from 2\textsuperscript{+} onward). Before this exposure time, the \( n\textsubscript{CorH\textsuperscript{+}} \) and \( n\textsubscript{CorH\textsuperscript{2+}} \) signals are very weak, and the ratio is uncertain. We can therefore assume that for these measurements \( d/dt(n\textsubscript{CorH\textsuperscript{+}}/n\textsubscript{CorH\textsuperscript{2+}}) = 0 \) and \( d/dt(n\textsubscript{CorH\textsuperscript{3+}}/n\textsubscript{CorH\textsuperscript{4+}}) = 0 \). The expressions for the CorH\textsuperscript{+} to CorH\textsuperscript{2+} as well as for the CorH\textsuperscript{3+} to CorH\textsuperscript{4+} energy barriers can then be written as:

\[
E_2 = -k_B T_{\text{gas}} \ln \left( \frac{A_3}{A_2 + \frac{n_{\text{CorH\textsuperscript{+}}}}{n_{\text{CorH\textsuperscript{2+}}}}} \right) \quad (5)
\]

\[
E_4 = -k_B T_{\text{gas}} \ln \left( \frac{A_3 + A_4 \frac{n_{\text{CorH\textsuperscript{3+}}}}{n_{\text{CorH\textsuperscript{4+}}}}}{A_4 + \frac{n_{\text{CorH\textsuperscript{3+}}}}{n_{\text{CorH\textsuperscript{4+}}}}} \right). \quad (6)
\]

From these expressions, we derive the energy barrier \( E_2 \) as 72 \pm 6\textsuperscript{+} meV and \( E_4 \) as 43 \pm 8\textsuperscript{+} meV, as shown in Figure 4(c). This shows that hydrogenation barriers are decreasing with increasing hydrogenation. However, our results also show that odd hydrogenated states dominate the mass spectrum even for high degrees of hydrogenation (Figure 3). This highlights the presence of a barrier–no-barrier alternation from one hydrogenated state to another, up to high hydrogenation states. Our results indicate that even if the hydrogenation barriers decrease for the first hydrogenations, they do not vanish completely and remain at higher hydrogenation states. The barriers derived in our study are similar to the one calculated by Rauls & Hornekær (2008) for neutral coronene. This means that the first hydrogenations of coronene cations should be comparable to the hydrogenation of neutral coronene. However, for a higher degree of hydrogenation we show that these barriers still exist, while the calculations from Rauls & Hornekær (2008) predict that these barriers vanish after a few hydrogenations. Recent mass spectrometric measurements of coronene films exposed to H/D atoms do not show preferences for even or odd hydrogenation states of neutral coronene (Thrower et al. 2012). However, these measurements are not very sensitive to barrier heights well below 100 meV, since the experiments were performed with atoms at beam temperature of 170 meV.

In PDRs exposed to UV fields less than few hundreds \( G_0 \), the spatial distribution of H\textsubscript{2} and PAHs does correlate (Habart et al. 2003, 2005; Compiègne et al. 2007), contrary to what is seen in the presence of strong UV fields (Tielens et al. 1993; Berné et al. 2009). The H\textsubscript{2} formation rates have been derived for several PDRs exposed to various UV radiation fields. These rates can be explained by the contribution of PAHs to the formation of H\textsubscript{2} (Habart et al. 2004). Depending on the UV intensity, the PAHs observed can be either PAH cations, which are present in regions at low visual extinctions \( A_V \), or neutral PAHs, which are located at higher extinctions. Wolfire et al. (2008) and Spaans & Meijerink (2005) have shown that high-UV and high-density PDRs \((n_H \geq 10^3 \text{ cm}^{-3} \) and \( G_0 \geq 100 \), \( G_0 = 1.6 \times 10^{-3} \text{ erg cm}^{-2} \text{ s}^{-1} \)) can maintain a \sim 30\% cationic fraction up to a few mag in \( A_V \). More relevant to this work, Cox & Spans (2006) have studied low-UV PDRs \((G_0 \lesssim 100)\), and followed the PAH charge balance for different densities, UV radiation fields, and metallicities. They found that PAH cations dominate over neutrals and anions for \( A_V \sim 2 \) mag. The H\textsubscript{2} formation rates observed in PDRs exposed to different UV fields can therefore be partly attributed to neutral and cationic PAHs.

Our results show that the hydrogenation processes of neutral and cationic PAHs are similar and should contribute similarly to the formation of H\textsubscript{2}. Further experimental investigations will allow us to derive the H\textsubscript{2} formation rate for PAH cations.

4. CONCLUSIONS

We have investigated the addition of hydrogen atoms to coronene cations in the gas phase and observed increasing hydrogenation with H exposure time. Our results show that odd hydrogenated states dominate the mass spectrum, which evidences the presence of a barrier for the further hydrogenation of odd hydrogenation states. The first hydrogen sticks to the coronene cations without a barrier (Snow et al. 1998; Hirama et al. 2004). The second and fourth hydrogenations are associated with barriers of about 72 \pm 6\textsuperscript{+} meV and 43 \pm 8\textsuperscript{+} meV, while the third and fifth hydrogenation are barrierless. These barriers are similar to the one calculated for neutral coronene (Rauls & Hornekær 2008). Our results indicate that superhydrogenated PAH cations (Li & Draine 2012) should also be found in the ISM, and be important catalysts for the formation of H\textsubscript{2}, as it is the case for their neutral counterparts.

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Near-Edge X-ray Absorption Mass Spectrometry of a Gas-Phase Peptide

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ABSTRACT: We have studied the dissociation of the gas-phase protonated peptide leucine enkephalin [YGGFL+H]+ upon X-ray absorption in the region of the C K-edge. The yield of photodissociation products was recorded as a function of photon energy. The total photoabsorption yield is qualitatively similar to near-edge X-ray absorption fine structure (NEXAFS) spectra recorded from condensed phase peptides and proteins. Fragment specificity reveals distinct qualitative differences between spectra obtained for different masses. Fragmentation channels can be assigned to specific electronic transitions of which are site-specific. For instance, C 1s → π* excitations in the leucine enkephalin aromatic side chains lead to relatively little fragmentation, whereas such excitations along the peptide backbone induce strong fragmentation.

INTRODUCTION

Soft X-rays of high flux and high resolution as provided by state of the art synchrotron facilities are an established tool for spectroscopic investigations of various properties of biomolecular systems. Near-edge X-ray absorption fine structure (NEXAFS) and X-ray photoelectron spectroscopy (XPS) are for instance employed to investigate thin films of peptides and proteins. Making use of a comprehensive NEXAFS data set for common amino acids at all relevant absorption edges, it could be shown that soft X-ray spectroscopy is a suitable tool for protein identification and mapping. Furthermore, NEXAFS spectral features characteristic for the peptide bond and for different peptide conformations have been identified. However, the sensitivity of NEXAFS to protein secondary structure seems weak.

Until very recently, soft X-ray spectroscopy was limited to biomolecular systems in the condensed phase with the exception of comparably small systems (nucleobases, amino acids etc.) for which gas-phase studies have been performed. A major advantage of gas-phase studies is the inherent absence of radiation damage, which can be a serious limitation in the condensed phase. Thin films of the amino acids glycine are for instance subject to peptide bond formation upon soft X-ray absorption, whereas dry DNA is found to be efficiently degraded by soft X-rays. Another advantage of gas-phase studies is the possibility of studying photoinduced molecular dynamics in the absence of an energy-dissipating environment. By using nanosolvated gas-phase systems the coupling to the environment can even be studied in detail. In a series of experimental soft X-ray spectroscopy studies on different gas phase amino acids, electronic transitions were assigned to the various spectral features and conformational effects were investigated. Similar studies have been performed for a variety of pyrimidines. Huels et al. investigated photofragmentation of DNA and RNA sugars upon photoabsorption at the C and O 1s edges by means of a photoelectron–photon coincidence technique and found extensive fragmentation.

Gas-phase studies have delivered a wealth of knowledge on small biomolecular systems. The step to larger biomolecular systems in the gas-phase however is difficult, as only a few specific small peptides such as glycyl-glycine (GG14) can be evaporated without thermal decomposition. A strategy to circumvent thermal decomposition is the use of small cyclic peptides, which possess high stability owing to their ring structure. However, most large biomolecular systems such as peptides, proteins, or DNA are so fragile that they disintegrate upon evaporation. The same holds for many smaller systems such as most amino acids. A number of experimental techniques have been developed to overcome this problem. Desorption techniques such as matrix-assisted laser desorption ionization (MALDI) or laser-induced acoustic desorption (LIAD) have already been used to generate gas-phase biomolecular targets for photoionization experiments. A versatile approach that at the same time allows for production of very pure targets is ESI. We have shown that radiofrequency (RF) trapping of mass-selected electrosprayed protonated peptides or oligonucleotides can provide targets.
sufficiently dense for vacuum ultraviolet (VUV)\textsuperscript{19,20} photofragmentation studies. Similar tandem mass spectrometry experiments on VUV photoionization of much larger protonated proteins\textsuperscript{21} and on deprotonated peptides\textsuperscript{22,23} have been reported recently. In a pioneering soft X-ray absorption study, Milosavljevic et al. used a trapped gas-phase target to obtain single and double C, N, and O near-edge photoionization yields of multiply protonated cytochrome c proteins.\textsuperscript{24} Non-dissociative ionization was found to be the dominant channel. (Note that for effusive targets of much smaller gas-phase molecules, X-ray partial-ion-yield spectroscopy has been performed earlier, for instance on CF\textsubscript{3} at the C and F K-edges.\textsuperscript{25})

In the present article, we present to our knowledge the first partial-ion-yields of a protonated peptide, the neurotransmitter leucine enkephaline ([\text{YGGFL+H}]\textsuperscript{+}, \textit{m} = 556.62 Da), upon soft X-ray absorption at the C K-edge. Peptides differ from proteins solely in size. In contrast to the much larger protein cytochrome c, leucine enkephaline is subject to extensive fragmentation upon soft X-ray absorption and is thus ideally suited to study the photon energy dependence of the various fragmentation channels. In view of the mass-spectrometric approach employed here, throughout this article we will therefore refer to the experimental technique as near-edge X-ray absorption mass spectrometry (NEXAMS). On the basis of the experimental data, fragmentation channels can be correlated to electronic transitions. There is a pronounced dependence of peptide stability on the soft X-ray absorption site.

### EXPERIMENTAL TECHNIQUE

We have interfaced a home-built tandem mass spectrometer with the soft X-ray beamline U49/2-PGM-1 at the BESSY II synchrotron facility (Berlin, Germany). Experimental details can be found in previous articles.\textsuperscript{19} Briefly, a beam of protonated peptides was extracted from an electrospray ionization (ESI) source, phase-space compressed by means of an RF ion funnel, and an RF quadrupole ion guide and subsequently mass selected using a RF quadrupole mass filter. Eventually the biomolecular cations were accumulated in a three-dimensional (3D) RF ion trap (Paul trap) to reach sufficient target density. Pulsed He buffer gas was employed to collisionally cool the trapped protonated peptides. The target molecules were then exposed for up to 2600 ms to the soft X-rays until about 5% of the protonated peptides in the trap were photoionized, resulting in the loss of the respective parent ions from the trap. Subsequent pulsed extraction of the cationic trap content into a linear time-of-flight (TOF) spectrometer (\textit{M}/\textit{ΔM} \approx 200) was used to obtain the mass spectrum of the photofragments. Note that ions with \textit{m}/\textit{z} < 70 are not trapped, and the mass spectra are thus cut off in this range. Due to carbon-rich contaminations of the monochromator gratings, at photon energies between 282 and 310 eV the stepsize was adapted to the anticipated spectral features, ranging from 0.25

Because native trap content and soft X-ray photon flux are known parameters, the loss of parent molecules from the trap can then be used to determine the relative photoionization cross section. Typical mass spectra for soft X-ray photofragmentation of protonated leucine enkephalin [\text{YGGFL+H}]\textsuperscript{+}, which contains the aliphatic amino acids guanine (G) and leucine (L) and the two aromatic amino acids tyrosine (Y) and phenylalanine (F), are displayed in Figure 1b. The spectra have been obtained at three different photon energies labeled A, B, and C corresponding to 285.5, 288.5, and 292.0 eV, respectively. We have systematically recorded such mass spectra for photon energies between 282 and 310 eV. The stepsize was adapted to the anticipated spectral features, ranging from 0.25

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (a) Schematic structure of leucine enkephalin. (b) Photofragmentation mass spectra for \textit{hv} = 285.5 eV (A), 288.5 eV (B), and 292 eV (C). The intensities in the mass region between 150 and 300 are multiplied by 5 and in the region between 300 and 560 by 10. Note, that the precursor does not appear as a positive peak, because the difference between initial trap content and photoionized trap content is displayed. (c) Typical C K-edge photoionization yield for the fragment with \textit{m}/\textit{z} 120 (phenylalanine immonium ion). The dominating spectral features are labeled A, B, and C. Indicated are the C 1s ionization energies for ring (\text{C}_{\text{ring}}),\textsuperscript{12} amino group (\text{CH}_3), peptide bond (\text{C} \equiv \text{O}) and carboxyl (\text{COOH}) sites.\textsuperscript{14}
eV to a few electronvolts at an energy resolution of 280 meV fwhm.

Peak integration in these mass spectra allowed us to determine cation yields as a function of photon energy. Integration windows were adjusted to the peak broadening with increasing mass. Identical integration windows were used for a given m/z over the whole photon energy range under study. Figure 1c displays such a cation yield for m/z 120, i.e., the phenylalanine (F) immonium ion. In the course of this article we will refer to such plots as NEXAMS spectra.

## RESULTS AND DISCUSSION

### General Spectral Features.

As mentioned in the last paragraph, this work is based on two types of spectra. Mass spectra of the photofragmentation products were recorded systematically for a large number of photon energies (I). NEXAMS spectra for various photofragments were then extracted from this data (II).

(I). **Photofragmentation Mass Spectra.** As is obvious from Figure 1b, the photofragmentation mass spectra are dominated by fragments with m/z 80–150 u. It is clear that virtually no fragments with masses exceeding m/z 300 are observed. For reference, Table 1 summarizes all fragments mentioned in this table.

<table>
<thead>
<tr>
<th>m/z</th>
<th>label</th>
<th>explanation</th>
<th>peak ratio A/B</th>
<th>peak ratio A/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td>phenylalanine/tyrosine immonium fragment</td>
<td>1.05 ± 0.03</td>
<td>0.9 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>phenylalanine/tyrosine immonium fragment</td>
<td>1.00 ± 0.01</td>
<td>1.1 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>tyrosine immonium fragment</td>
<td>1.18 ± 0.02</td>
<td>1.4 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>F phenylalanine immonium</td>
<td>1.22 ± 0.02</td>
<td>1.8 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>Y tyrosine immonium</td>
<td>1.23 ± 0.03</td>
<td>2.3 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>a2–107 N-terminal fragment + tyrosine side chain loss</td>
<td>1.35 ± 0.02</td>
<td>2.6 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>b2–107 N-terminal fragment + tyrosine side chain loss</td>
<td>1.54 ± 0.18</td>
<td>1.9 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>131</td>
<td>c2–107 N-terminal fragment + tyrosine side chain loss</td>
<td>1.51 ± 0.03</td>
<td>2.2 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>171</td>
<td>b2–107 N-terminal fragment + tyrosine side chain loss</td>
<td>1.78 ± 0.18</td>
<td>5.1 ± 1.22</td>
<td></td>
</tr>
<tr>
<td>161</td>
<td>GF-28 internal fragment</td>
<td>1.18 ± 0.24</td>
<td>3.7 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>177</td>
<td>GF-28 internal fragment</td>
<td>1.12 ± 0.15</td>
<td>2.7 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>205</td>
<td>GF internal fragment</td>
<td>1.39 ± 0.06</td>
<td>5.5 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>221</td>
<td>b2 N-terminal fragment</td>
<td>1.16 ± 0.06</td>
<td>4.3 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>278</td>
<td>b2b2(M+H)+ doubly charged parent dominates</td>
<td>1.7 ± 0.18</td>
<td>8.9 ± 1.17</td>
<td></td>
</tr>
<tr>
<td>295</td>
<td></td>
<td>1.03 ± 0.20</td>
<td>6.5 ± 0.93</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1b have previously only been observed in keV ion-induced dissociation (KID). The predominance of immonium ions in KID and their abundance in VUV photofragmentation has been attributed to relatively high excitation energies in combination with fast dissociation preceded by charge migration.

A second class of fragments was previously observed for VUV photoionization as well: The spectra in Figure 1b exhibit a series of fragments due to backbone scission (a2, b2, b3, and c2) which have subsequently cooled off excess energy by losing the tyrosine side chain with m/z 107 u. The structures of these fragments can be found in Figure 3. A number of heavier fragments is observed, that are commonly found in conventional mass spectrometric studies. These include an internal GF fragment with m/z 20528 and a (GF-CO) fragment with m/z 177. These fragments as well as m/z 295 are probably derived from a cyclicly rearranged a2 fragment of leucine enkephalin. Another relatively strong larger fragment is found at m/z 221. Finally, the peaks at m/z 278 and 279 due to the b2 and y2 fragments, respectively (see sketch in Figure 3), cannot be separated in the spectrum. The [YGGFL+H]+22 dication can also contribute to this peak.

(II). **NEXAMS Spectra.** The overall shapes of the NEXAMS distributions for the various fragments are very similar. Therefore, the spectral features will be discussed exemplarily using the spectrum displayed in Figure 1c. Clearly, the spectrum is dominated by two sharp peaks labeled A and B at 285.4 and 288.4 eV with full widths at half-maximum (fwhm) of 0.6 and 0.7 eV, respectively. A broader structure (C) starts approximately at 290 eV and extends far beyond 300 eV. The spectra show some resemblance to single photoionization yields of the large, multiply protonated protein cytochrome c (m ≈ 12834 Da). Nevertheless, for cytochrome c the broad structure C is by far the strongest spectral feature.

NEXAFS spectra of condensed phase proteins and peptides can be well predicted by summation of the spectra of their constituent amino acids if a correction for the lack of the peptide bonds is taken into account (C, N, and O 1s NEXAFS spectra for 22 amino acids including G, L, Y, and F can be found in ref 1). It is interesting to note that the condensed phase NEXAFS spectra resemble the spectra displayed in Figure 2 closer than the gas-phase protein data.

To assign transitions to the different peaks in the (gas-phase) NEXAMS spectra, it is straightforward to employ the available NEXAFS data on gas-phase amino acids. More recently, such gas-phase data became available for Y and F11 and for G.4 In the basis of those results, the main features of our NEXAMS spectra can be assigned. Peak A (285.4 eV) is due to C 1s excitations into the π* orbitals of the Y and F aromatic rings. The fact that at this photon energy solely resonant transitions in the aromatic side chains are induced is a very interesting feature as it adds site specificity to the data. Peak B (288.4 eV) stems from transitions of C 1s electrons into the π* orbitals of the amid group and is nonspecific regarding the constituent amino-acids. On the other hand, the weak structure on the low-energy side of peak B, which is recognizable in some spectra hints at a contribution of C 1s excitations in the Y and F aromatic rings. These energetic positions of the resonances are in very good agreement with existing data, for instance, for gas-phase multiply protonated cytochrome c (A: 285.5 eV; B: 288.5 eV4), the gas-phase neutral dipeptide glycyglycine (B: 288.4 eV14), gas-phase tyrosine (A: 285.5 eV; B: 288.5 eV11), and thin films of G, GG, GGG, and fibrinogen (B: 288.2–288.6 eV).
The C 1s ionization energies for the peptide backbone and termini can be assumed to be similar to those of the glycyl-glycine dipeptide determined experimentally by Feyer et al.\textsuperscript{14} as 292.32 eV (C bonded to amino groups), 293.85 eV (peptide bond C), and 295.37 eV (carboxyl terminal C). In addition, the (lower) ionization energies of the aromatic side chains have been determined experimentally for the isolated amino acids to be 290.2 eV (Y) and 290.3 eV (F),\textsuperscript{11} which is exactly at the onset of the broad structure C (see Figure 1). There is no gas-phase data available for leucine, but it can be assumed that the C 1s ionization energy of the leucine side chain has a value similar to the 291.0 eV found for alanine, the simplest aliphatic amino acid.\textsuperscript{10} A substantial fraction of C is thus due to K-shell ionization, which, in light elements such as C, rapidly decays nonradiatively by Auger de-excitation.\textsuperscript{32} In this regime, a triply charged \{YGGFL+H\}\textsuperscript{3+} intermediate is thus formed, whereas below the ionization threshold, resonant Auger decay leads to the formation of doubly charged \{YGGFL+H\}\textsuperscript{2+} intermediate ions. Intact \{YGGFL+H\}\textsuperscript{3+} is clearly absent in the observed mass-spectra, and to our knowledge has also not been observed in other studies. For \{YGGFL+H\}\textsuperscript{2+} a small yield cannot be ruled out. A second class of contributions to C is due to various C 1s σ★- and Rydberg transitions. Of particular interest is a broad continuum resonance at ∼293.5 eV due to σ★-resonances in the aromatic rings.\textsuperscript{11} In the high energy tail of C, mainly shape resonances contribute.\textsuperscript{11,14}

**Fragment Specificity.** In a VUV photodissociation study of the \{YGGFL+H\}\textsuperscript{+} precursor, we have shown that the loss of

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**Figure 2.** C K-edge NEXAMS spectra of \{YGGFL+H\}\textsuperscript{+} for Y and F immonium ions and related fragments of (right) intact Y (d) and F (e) immonium ion and (left) related fragments common to both amino acids (a, b). The sketches next to the spectra display the structure of the respective fragment in the parent molecule. Intensities are given in arbitrary units on the same relative scale as the other NEXAMS spectra in this article.

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**Figure 3.** C K-edge NEXAMS spectra of \{YGGFL+H\}\textsuperscript{+} for fragments stemming from backbone scission followed by Y-side chain loss. Intensities are given in arbitrary units on the same relative scale as the other NEXAMS spectra in this article.
the aromatic Y and F side chains is a dominating process.19 As the immonium ions of the aromatic amino acids Y and F and their fragments play an important role in the fragmentation of [YGGFL+H]+, Figure 2 displays NEXAMS data for cationic fragments with m/z 136 and 120 (immonium ions of Y and F, respectively), m/z 107 (fragment of the Y immonium ion) and m/z 91 and 77 (common fragments of Y and F immonium ions). Qualitatively, the spectra are similar, which implies that near the C 1s edge X-ray absorption anywhere in [YGGFL+H]+ can trigger the formation of these fragments. This is in line with the results obtained in the VUV photofragmentation study of [YG,F+H]+, which proved that side chain loss occurs independently of the initial photoabsorption site, a finding that can be explained in a scenario of charge migration toward the aromatic side-chain followed by fast bond scission.20

There are, however, clear quantitative differences between the immonium-ion related fragment data. It is convenient for the discussion to quantify these differences in the form of the peak ratios as summarized in Table 1. Here, A/B denotes the ratio of the respective peak integrals (background subtracted), whereas A/C is the ratio between the peak intensity in A and the average intensity between 290 and 300 eV. As mentioned before, peaks A and B are due to C 1s → π* excitations in the aromatic rings and in the amide groups, respectively.

From Table 1 it is obvious that the immonium ions at m/z 136 and 120 are more likely to be formed upon 1s → π* excitations in the Y and F aromatic rings than along the peptide backbone because A/B = 1.23 and 1.22, respectively. C 1s ionization is clearly less efficient in forming these fragments than excitation as indicated by A/C values of 1.8 and 2.3, respectively. Note that formation of the nonterminal immonium ion m/z 120 requires two bond scissions, whereas formation of m/z 136 requires disruption of a single bond, only. With decreasing size of immonium fragments, A/C decreases rapidly, i.e., ionization becomes more dominant. For m/z 77, A/C = 0.9. This decrease is intuitively expected as higher initial charge and/or excitation energy in the system usually shifts the fragmentation pattern toward smaller masses.33 For immonium ion fragments in particular, it is generally assumed that they are formed from the respective immonium ion by loss of a neutral fragment, implying an additional bond scission.34 It is remarkable that A/B decreases for m/z 91 and 77 to values close to 1. Apparently, the C 1s → π* excitation in one of the six-membered rings is thus not more likely to induce formation of a small aromatic fragment than photoabsorption along the peptide backbone.

Complementary information can be obtained from the NEXAMS data for the fragmentation channels involving backbone scission. These channels are usually associated with lower activation energies than formation of immonium ions and immonium ion fragments because scission of fewer or weaker bonds is involved. For all lower activation energy channels studied here, C 1s → π* excitation in one of the six-membered rings has the strongest contribution. We can classify these channels into two groups. As mentioned previously, a number of fragments observed here and also earlier in KID and VUV photofragmentation are due to backbone fragmentation preceded by loss of a m/z 107 Y side chain. The respective NEXAMS data for the four strongest fragments are displayed in Figure 3. The sketch shows which bonds need to be broken in order to form the different fragments. Clearly, for the relatively small a1−107, b1−107, and c1−107 fragments, almost identical NEXAMS data are observed. (We note that the a1−107 fragment at m/z 86 may in part also be due to the L immonium ion.) For these fragments, the ratios A/B and A/C are much larger than 1, namely, ∼1.5 and ≥1.9 (cf. Table 1). This implies that production of N-terminal fragments that lost the 107 side chain is mostly driven by 1s → π* excitations on one of the aromatic rings. For the larger b1−107 fragment, the effect is most pronounced.

The NEXAMS data for various larger fragments formed without loss of the m/z 107 side chain is displayed in Figure 4. Here, A/B slightly exceeds unity but A/C has very large values. The large fragment thus mainly result from C 1s excitation and not from ionization. In the ionization case, probably too much energy remains in the molecule to allow for survival of the larger fragments.

Figures 3 and 4 together confirm a conclusion based on our recent VUV photofragmentation study on [YGGFL+H]+,19 the loss of the m/z 107 tyrosine side chain efficiently cools off excess energy. Fragments a2, b2, and c2 can be formed even for above-threshold X-ray absorption if their formation is accompanied by side chain loss. Other relatively large fragments without a loss of the Y side chain appear not to be formed above the threshold.

For m/z 278 and 279, which is attributed to the [YGGFL+H]+2+ dication, the b2 and/or the γ2 fragment (see Figure 4), low intensities lead to large error bars. It is, however, clear that this spectrum is special in the sense that A is by far the dominating peak, whereas B is weaker and C is almost absent. Clearly, mainly C 1s → π* excitations on the aromatic rings are related to this channel. The particularly high A/B ratio might indicate that for this peak the nondissociative ionization channel contributes strongly and that formation of an intact protonated dication is most likely when the photon is absorbed by one of the aromatic side chains.
CONCLUSION

In this article we have presented a NEXAMS study for a gas-phase protonated peptide, the neurotransmitter leucine enkephalin. Partial-ion-yield for various fragments were systematically investigated at the C K-edge as a function of photon energy. Qualitatively, the observed NEXAMS data resembles, e.g., NEXAFS spectra of peptides and proteins in the condensed phase and photoionization yields of multiply protonated proteins in the gas phase. Quantitatively, significant differences are observed. The observed spectral features in NEXAMS data can be assigned to a number of inner shell excitations and ionizations localized at different sites within the peptide.

The resonance at 284.4 eV is solely due to C 1s → π* excitations in the aromatic rings. These excitations predominantly lead to the formation of large fragments up to the intact protonated dication and are weaker in the case of small fragments. Thus, C 1s → π* excitations in the aromatic rings are apparently the softest X-ray absorption channels in protonated leucine enkephalin. However, C 1s excitation along the peptide backbone contributes strongly to the protonated leucine enkephalin. However, C 1s excitation is apparently the softest X-ray absorption channels in protonated leucine enkephalin. However, C 1s excitation along the peptide backbone contributes strongly to the formation of smaller fragments and is a more destructive channel. C 1s ionization (above threshold) leads to very extensive fragmentation, independently of the actual absorption site.

From the presented data it is clear that NEXAMS is a new feasible approach to obtain information on dynamical processes in intermediate sized biomolecular systems. Together with the fact that site-selective resonances can be identified, this implies the possibility of studying charge migration upon inner shell excitation in synthetic model systems.

Judging from the extensive fragmentation observed in [YGGFLH]+ upon soft X-ray absorption, the present study is focused on the high excitation regime. On the other hand, Milosavljevic et al. have investigated a large protein where virtually no fragmentation is observed. Therefore, it will be interesting to explore the intermediate regime where excitation energies become just sufficient to cause fragmentation. In this threshold region, site selectivities might be even more pronounced. NEXAMS is an experimental technique ideally suited for such studies.

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Notes

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REFERENCES


Statistical fragmentation of doubly charged anthracene induced by fluorine-beam impact at 3 keV

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The fractional scheme of the doubly charged anthracene molecule (C14H102+) has been studied via monocharged fluorine impact at 3 keV using the CIDEC method (collision-induced dissociation under energy control). Doubly or singly charged fragments resulting from the loss of neutrals (H, C2H2) or CxHn+ (x = 1–5; y = 1–5) have been measured versus the excitation energy of the parent molecule C14H102+. The branching ratio of the CxHn+ emission process was found to be 16%. For the major neutral evaporation channels via nH or nC2H2 (n = 1, 2) emission, the measured population distributions have been fitted using a Rice-Ramsperger-Kassel–Kassel (RRK) statistical dissociation and cascade model. The simulated rate-energy dependences of the two primary competing pathways, i.e., the loss of H or C2H2 from C14H102+, present a crossing point at 13 eV. This feature is characteristic of two different fragmentation mechanisms: a direct cleavage for the loss of H for energy above the crossing point and a rearrangement process for the loss of C2H2 below the crossing point.

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I. INTRODUCTION

The abundant presence of polycyclic aromatic hydrocarbons (PAHs) in the interstellar medium [1] has initiated numerous laboratory studies of this group of molecules. For about two or three decades, numerous works have been devoted to detailed studies of their electronic properties, (in-)stabilities, and structures [2–6]. These data were used as input for various models to estimate, for example, the PAH distributions and lifetimes in particular interstellar regions where the UV light is intense [7,8] or and where keV ions, such as H+ or He+, are present [9]. The dissociation dynamics of PAHs upon a variety of excitation mechanisms have been also studied extensively. In many laboratory studies on PAH dissociation a mass spectrometric approach is employed, i.e., the fragmentation patterns of gas phase PAH molecules upon single ionization and excitation by electron bombardment, multiple collisional activation steps [10], nanoparticle and femtosecond laser pulse [11], or synchrotron radiation [12,13].

The prevalent decay pathways for such species have been found to correspond to the loss of H and C2H2 molecules, almost independently of the size of the molecule [10–12,14]. In the case of anthracene (C14H10), a small three-ringed PAH, unimolecular dissociation of C14H10+ has been studied by time-resolved vacuum ultraviolet (VUV) photoionization combined with Rice–Ramsperger–Kassel–Marcus (RRKM) modeling [14]. Regarding PAH molecules at higher charge state, it has been argued that in the interstellar medium, physical conditions of certain regions permit their formation [3]. In the so-called H II regions, where the energy of the present UV photons is less than 13.6 eV, PAH dications can be formed and heated by sequential two-photon absorption. Nevertheless, until now, very few investigations have been devoted to studying the fragmentation of multicharged PAH. In a recent publication, Holm et al. have performed density functional theory calculations to determine the ionization potentials and the dissociation energies of multiply charged PAHs including C14H10q+ (q ≤ 6) [9]. In collision experiments using keV protons and α particles, multifragmentation of C14H10q+ with q up to 3 has been reported and the excitation energy deposited by the ions in an anthracene molecule has been interpreted as due to electronic stopping and simulated with the SRIM software [15].

In this paper, we report on an experimental study of the dissociation pattern of doubly charged anthracene molecules prepared in collisions between a singly charged fluorine ion beam at 3 keV and a neutral molecular jet. As compared to previous collision experiments between ions and PAHs, the breakthrough of this work is to provide a precise determination of the charge as well as the excitation energy of the parent ions prior to the dissociation. For the dissociation of C14H102+, two types of processes are distinguished: the evaporation process for the successive loss of small neutrals and the asymmetrical fission process, also referred to as charge separation reaction, involving the loss of a small singly charged fragment. The measured population distributions for the main evaporation channels, i.e., the loss of nH and the loss of nC2H2 (n = 1, 2), have been fitted using a simple Rice–Ramsperger–Kassel (RRK) statistical and cascade model. Dissociation energies and pre-exponential factors for the parent C14H102+ ions and the intermediate precursory ions (C14H10-H)+ and (C14H10- C2H2)+ are reported. The energy dependencies of the two primary competing pathways, i.e., the loss of H or C2H2 from C14H102+, are discussed.

The experimental method used in this work is the CIDEC method (collision-induced dissociation under energy control), capable of providing the internal excitation energy map of the target molecules prior to dissociation. This method originated from the double-charge-transfer (DCT) spectroscopy developed for studying energy levels of electronic states of doubly charged molecular ions [16]. DCT spectroscopy is based on the formation of negative ions by double electron capture in inelastic collisions between singly charged incident projectiles A+ and neutral molecular targets M, A+ + M → A− + M2++. By measuring the kinetic energy loss of the anion.
A−, the first excitation energy levels of the doubly charged target could be determined. The CIDECE method developed in our laboratory combines the DCT spectroscopy, fragment mass spectroscopy, and coincidence measurement techniques [17]. It is aimed at studying the decay of target molecules as a function of the excitation energy deposited during ion-target collisions. In the past years, several molecules have been studied with this method, such as C60, adenine, deoxyribose, and W(CO)6 [17–20]. In the present work, doubly charged anthracene ions were prepared via the double-charge-transfer process in the following collisions:

\[ \text{F}^+ + \text{C}_{14}\text{H}_{10} \rightarrow \text{F}^- + \text{C}_{14}\text{H}_{10}^{2+}. \]

Depending on the projectile ion and on the collision velocity, primarily electronic or nuclear excitation can occur. In our case, fluorine-ion impact at 3 keV kinetic energy, the energy deposition is mainly due to the electronic interaction. Therefore, the excitation mechanism in the reaction (1) is comparable to photon excitation using UV synchrotron radiation. Nevertheless, the main interest of the CIDECE method is that the electrons are not ionized to the continuum but transferred to the projectile occupying the ground state of F− at a well-known energy level. As a consequence, we are able to measure the excitation energy of the parent ions in a larger energy range (0–30 eV) instead of being limited in the vicinity of the appearance energies of dissociation channels. This allows us to provide a better comparison between the experimental data and the theoretical model.

II. EXPERIMENT SETUP

The experimental setup, depicted in Fig. 1, has been described in previous papers [20,21]. A monocharged fluorine ion beam extracted at 3 kV from an ECR (electron cyclotron resonance) Nanogan III ion source collided on a neutral beam extracted at 3 kV from an ECR (electron cyclotron resonance) Nanogan III ion source. The F+ ion beam was focused with an Einzel lens into the interaction region to about 0.2 mm in diameter with an energy dispersion estimated to be 1 eV. First, this primary beam was selected by the electrostatic analyzer and collected with a Faraday cup (FC) placed behind the exit slit (opening: 0.2 mm). Then, all the electrostatic and magnetic elements of the beam line, especially the Einzel lens, were adjusted to optimize the energy resolution. After the primary beam adjustment, the polarization of the electrostatic analyzer was reversed in order to analyze the kinetic energy of anions F− formed in the collisions. A channeltron facing the Faraday cup was turned on to detect the secondary electrons produced at the impact of the F− anions on the Faraday cup. The recoil ions C14H10+ or charged fragments were extracted by a transverse electric field of 50 V/cm from the collision region. The transverse extension of the incident F+ ion beam along the extraction field induced additional energy dispersion for the scattered F+ ion beam estimated to be 1 eV. After the extraction, the recoil ions were accelerated to about 3 keV per charge and analyzed with a time-of-flight (TOF) spectrometer. The time for C14H10+ to fly out of the extraction field was about textr = 1.1 μs. This duration determined the time window for the dissociation processes studied in this work. For each event, the detection of the negative ion F− provided the reference trigger for the time-to-digital converter of the TOF spectrometer. As a consequence, the TOF of the fragments resulting from a collision event was recorded in coincidence with the detection of the corresponding scattered projectile F−.

The kinetic energy of the scattered projectile anions F− was analyzed by scanning step by step the voltage applied to the electrostatic analyzer around the initial kinetic energy of the projectiles. At each step, the TOFs of doubly charged intact anthracene or fragments were recorded and plotted, together with the voltage of the analyzer, in a two-dimensional (2D) coincidence spectrum. The electrostatic analyzer scanning voltage was first converted, using the argon gas target as calibration reference, to the kinetic energy loss ΔE of F− (see Ref. [22] for details), and then to the deposited energy E_D in the anthracene target during the collision. Based on the principle of the CIDECE method, the deposited energy was estimated using the relation [18]

\[ E_D = \Delta E - \delta. \]

The energy defect δ of the collision (1) is defined as

\[ \delta = I_1(C_{14}H_{10}) + I_2(C_{14}H_{10}) - I_1(F^-) - A_1(F^+) \].

Knowing the first ionization potential and the electron affinity of the fluorine I_1(F) = 17.42 eV, A_1(F+) = 3.4 eV [23] and the first and second ionization potentials of the anthracene, I_1(C_{14}H_{10}) = 7.44 eV [24] and I_2(C_{14}H_{10}) = 11.8 eV [3], δ was calculated to be ~1.6 eV. Taking into account the initial energy of neutral anthracene at T = 320 K, the excitation energy of C_{14}H_{10}+ was calculated:

\[ E_{\text{exc}} = E_D + E_{\text{thermal}}. \]

E_{\text{thermal}} at 320 K was estimated to be 0.2 eV [7]. After the energy calibration, the TOF of doubly charged intact anthracene or fragments was plotted as a function of the excitation energy E_{\text{exc}} of the parent C_{14}H_{10}+ ions in a 2D-coincidence spectrum which will be called EX-RI spectrum (excitation-recoil ion) in the following.

III. RESULTS AND ANALYSES

The mass spectrum of recoil ions recorded in coincidence with projectile F− anions but without projectile kinetic energy analysis, i.e., integrated over the whole distribution of possible excitation energies, is presented in Fig. 2. In order to extract cross sections for different dissociation channels, only one
FIG. 2. TOF spectrum in F⁺ + C₁₄H₁₀ → F⁻ + C₁₄H₁₀⁺/$\Delta$E collisions calibrated to $m/q$ value of the detected ions. The molecular structure of anthracene is shown in the inset of the figure.

Other smaller peaks in Fig. 2 are assigned to the following decay schemes. The peak (C₁₄H₁₀-2C₂H₂)²⁺ corresponds to the successive emission of two neutral C₂H₂ fragments. The peak C₁₄H₁₀³⁺ is due to one electron loss in the reaction (1). This weak channel could be attributed to the postcollisional statistical ionization of hot C₁₄H₁₀²⁺ or to electron loss during the collision. From the experimental data of the present experiment, it is difficult to determine exactly the mechanism. The broad peaks measured for $m/q > 95$ are attributed to the detection of the heavy singly charged fragment of the asymmetrical fission process. The peaks for $m/q < 55$ are mainly attributed to a light singly charged fragment from asymmetrical fission in the cases where the heavy one was lost due to the limited collection and detection efficiency. A part of these small fragments is due to multifragmentation processes, i.e., the breakdown of C₁₄H₁₀⁺ into several small fragments. The mass, the assignment, and the yields for intact C₁₄H₁₀₂⁺ and (C₁₄H₁₀-2H)²⁺ are given in Table I. For asymmetrical fission processes, the number of hydrogen atoms in the fragments cannot be resolved precisely; hence the peaks are attributed to the expected most abundant fragment. For instance, the peak attributed (C₁₄H₁₀-C₂H₂)²⁺ includes also a minor contribution from (C₁₄H₁₀-C₂H₂)³⁺ due to the emission of C₂H₃⁺. Indeed, from the correlation spectrum the successive emission of two neutral C₂H₂ fragments.

FIG. 3. (Color online) (a) 2D spectrum in F⁺ + C₁₄H₁₀ → F⁻ + C₁₄H₁₀⁺/$\Delta$E collisions. Horizontal axis: $m/q$ of the recoil ions; vertical axis: excitation energy of the parent C₁₄H₁₀⁺ before dissociation. (b) Projection of the 2D spectrum to the horizontal axis. (c) Projection of the 2D spectrum to the vertical axis.

FIG. 4. (Color online) (a) An enlarged view of the 2D spectrum from Fig. 3(a). (b)–(e) Partial projection to the excitation energy axis of the main spots assigned respectively to C₁₄H₁₀⁺, (C₁₄H₁₀-2H)²⁺, (C₁₄H₁₀-C₂H₂)²⁺, and (C₁₄H₁₀-2C₂H₂)²⁺. The top scale and the bottom scale are converted respectively to the kinetic energy loss $\Delta E$ of the anions F⁻ and the excitation energy of the parent C₁₄H₁₀⁺ ions.
TABLE I. $E$: mean excitation energy of the $\text{C}_{14}\text{H}_{10}^{2+}$ parent ions determined by the center of the corresponding energy distribution curve. Appearance excitation energies $E_{\text{app}}$, count, and the attribution are presented for each dissociation channel.

<table>
<thead>
<tr>
<th>Mass</th>
<th>Charge</th>
<th>$E$ (eV)</th>
<th>FWHM (eV)</th>
<th>$E_{\text{app}}$ (eV)</th>
<th>Counts</th>
<th>Attribution</th>
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<td>8.5</td>
<td>4.6</td>
<td>682</td>
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<td>6.2</td>
<td>2807</td>
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</tr>
<tr>
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<td>2</td>
<td>10.4</td>
<td>5</td>
<td>4703</td>
<td></td>
<td>$(\text{C}<em>{14}\text{H}</em>{10}-\text{C}_{2}\text{H}^{2+})^{+}$</td>
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<tr>
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<td>2</td>
<td>13.5</td>
<td>4.8</td>
<td>782</td>
<td></td>
<td>$(\text{C}<em>{14}\text{H}</em>{10}-2(\text{C}_{2}\text{H})^{2+})^{+}$</td>
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<tr>
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<td></td>
<td>240</td>
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<td>344</td>
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<tr>
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<td></td>
<td>123</td>
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<td>$\text{C}<em>{14}\text{H}</em>{10}^{3+}$</td>
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</table>

between fragment ions (not shown here), the presence of the fission channel $\text{C}_{14}\text{H}_{10}^{2+} \rightarrow (\text{C}_{14}\text{H}_{10}-\text{C}_{2}\text{H})^{+} + \text{C}_{2}^{+}$ is confirmed. The correlation spectrum shows also that the most favorable fission channels correspond to the loss of $\text{C}_{2}\text{H}_{2}^{+}$ and $\text{C}_{2}\text{H}^{+}$. This is slightly different from the observation of Leach [3] who found a maximum for the $\text{C}_{2}\text{H}_{2}^{+}$ and $\text{C}_{2}^{+}$ loss. From the yields given in Table I, the total branching ratio of all fission channels has been estimated to be 16%; the branching ratios for the two main neutral fragment emission channels corresponding to $\text{C}_{2}^{+}$ or 2H loss have been found to be about 48% and 28%, respectively. This shows that the dominant dissociation process for $\text{C}_{14}\text{H}_{10}^{2+}$ is the emission of neutrals.

The one-dimensional spectrum of Fig. 2 was obtained by the total projection of the 2D EX-RI spectrum [Fig. 3(a)] to the horizontal axis. It is displayed again in Fig. 3(b) to help the assignment of the spots on the 2D spectrum. The total projection of the 2D spectrum to the vertical axis led to Fig. 3(c) corresponding to the whole excitation energy distribution of the $\text{C}_{14}\text{H}_{10}^{2+}$ parent ions prior to dissociation. We note that the most probable excitation energy is about 11 eV and that the energy distribution exhibits a long tail extending up to 30 eV. Figure 4(a) is an enlarged view of the excitation energy region from 10 to 14 eV, one-fragment loss channels corresponding to the loss of $\text{C}_{2}^{+}$, $\text{C}_{2}^{+}$, and $\text{C}_{2}^{+}$ are observed. The mean energy necessary for driving the loss of a charged $\text{C}_{2}^{+}$ fragment was found to be about 4 eV larger than for the loss of a neutral $\text{C}_{2}$. This indicates that the fission barrier in the above charge separation process is higher than the dissociation energy for the loss of the neutral. In the high-energy region (E > 13.5 eV), the loss of two fragments is observed via several competing channels. From 13.5 to 14 eV, the loss of $\text{C}_{2}$, 2$\text{C}_{2}^{+}$, or $\text{H}_{2}$, prevails, while at about 16 eV the loss of a charged fragment and a neutral is observed.

In order to compare the experimental data with a model (see Sec. IV), the measured energy distribution curves of Fig. 4 have to be corrected to account for peak broadening inherent to the apparatus and to the initial energy dispersion of the primary beam. Thus, all the experimental energy distributions have been deconvoluted using a Gaussian curve of 3.8 eV in full width at half maximum. This reference curve corresponds to the measured $F^{-}$ kinetic energy loss distribution obtained in collisions with the reference Ar gas target for which the intrinsic broadening of the populated state of $\text{Ar}^{2+}$ is considered as negligible. Figure 5 shows the corrected excitation energy distributions of $\text{C}_{14}\text{H}_{10}^{2+}$ for intact molecules and the main evaporation channels. The sum of these curves is also presented in Fig. 5. It gives approximately the total excitation energy distribution excluding the contribution of the relatively weak fission channels. The appearance excitation energies $E_{\text{app}}$ for the evaporation channels have been estimated from the rising edge of each distribution (Table I). In a time-resolved photoionization experiment, Ling et al. [14] have measured the $E_{\text{app}}$ of $(\text{C}_{14}\text{H}_{10}-\text{C}_{2}^{+})^{+}$ due to the loss of a $\text{C}_{2}$ from $\text{C}_{14}\text{H}_{10}^{+}$. The values $E_{\text{app}}$ = 15.2 and 14.2 eV were reported for a storage time of 24 $\mu$s and 5 ms, respectively. These measured $E_{\text{app}}$ values lead to appearance internal excitation energies $E_{\text{app}}$ = $E_{\text{app}}$ – $I_{1}$ + $E_{\text{th}}$ of 8.3 and 7.3 eV, respectively,
where $I_1 = 7.4 \text{ eV}$ is the first ionization potential of anthracene and $E_{th} \approx 0.5 \text{ eV}$ is the additional thermal ion energy in Ling’s experiment (453 K). Our estimation of $E_{\text{exc}}^{\text{app}} = 8 \text{ eV}$ for the emission of a $C_2H_2$ fragment from $C_{14}H_{10}^+$ compares favorably with the values of Ling et al.

IV. SIMULATION

In the following, the corrected excitation energy distributions are simulated using a simple statistical unimolecular dissociation and cascade model. Details of the method can be found in the papers on the fragmentation of $C_{60}^{4+}$ and $W(CO)_6^{2+}$ molecules [17,20] and only a short description is presented here. In a first approximation, we used a simplified fragmentation scheme shown in Fig. 6. The weak asymmetrical fission processes and minor evaporation decay channels such as the emission of $H$ from $(C_{14}H_{10}-C_2H_2)^{2+}$ or the emission of $C_2H_2$ from $(C_{14}H_{10}-H)^{2+}$ or $(C_{14}H_{10}-2H)^{2+}$ were not taken into account. Only the main evaporation channels, i.e., the loss of one fragment, $H$ or $C_2H_2$, and the successive loss of two fragments, $2H$ or $2C_2H_2$, were considered. Direct jumps to the final states $(C_{14}H_{10}-2H)^{2+}$ and $(C_{14}H_{10}-2C_2H_2)^{2+}$ via the loss of a $H$ or a $C_2H_2$ had been excluded in the modeling due to their unlikely contributions. In spite of the very low population of $(C_{14}H_{10}-H)^{2+}$, the H loss channel has to be included in the model because it is the precursor ion for the dominant 2H loss channel. As shown in Fig. 6, a rate constant for unimolecular dissociation was defined for each channel:

- $k_\alpha$ for channel ($\alpha$): $(C_{14}H_{10})^+ \rightarrow (C_{14}H_{10}-C_2H_2)^{2+} + C_2H_2$,
- $k_\beta$ for channel ($\beta$): $(C_{14}H_{10}-C_2H_2)^{2+} \rightarrow [C_{14}H_{10}-2(C_2H_2)]^+ + C_2H_2$,
- $k_\alpha'$ for channel ($\alpha'$): $(C_{14}H_{10})^+ \rightarrow (C_{14}H_{10}-H)^{2+} + H$,
- and $k_\beta'$ for channel ($\beta'$): $(C_{14}H_{10})^+ - (C_{14}H_{10}-2H)^{2+} + H$.

These rate constants depend sensitively on the excitation energy of the parent ions $C_{14}H_{10}^{2+}$ or the precursory transient parent ions $(C_{14}H_{10}-H)^{2+}$ and $(C_{14}H_{10}-C_2H_2)^{2+}$. They were supposed to follow the Arrhenius formula based on the Rice-Ramsperger-Kassel statistical model [25] and the recent work of J. U. Andersen et al. [26]:

$$k = A_d \exp(-E_d/k_BT_e).$$

In this relation, $k_BT_e$ stands for the Boltzmann constant, $E_d$ the dissociation energy, and $A_d$ the preexponential factor. The emission temperature $T_e$ of the molecule was obtained from the excitation energy $E$ using a linear approximation valid at high temperature that includes the so-called finite heat-bath correction [26] (>1000 K):

$$T_e = (E - E_d/2)/C.$$  

The heat capacity of anthracene, $C = 0.0053 \text{ eV/K}$, was taken from the NIST chemistry WebBook [27] at 1500 K. The aim of the simulation was to determine the values of the dissociation energy $E_d$ and the preexponential factor $A_d$ for each decay step.

The relative probabilities for $C_{14}H_{10}^{2+}$ to emit 1 H, 2 H, 1 $C_2H_2$, or 2 $C_2H_2$ inside a time window of $t_{\text{stat}} = 1.1 \mu$s were calculated using the cascade model [21,22] as a function of the initial excitation energy $E$ of the parent $C_{14}H_{10}^{2+}$ ions. The probability versus energy curves, which are referred to as breakdown curves in photoionization experiments [13], are displayed in Fig. 7. The simulated population distributions for $C_{14}H_{10}^{2+}$, $(C_{14}H_{10}-nH)^{2+}$ and $(C_{14}H_{10}-nC_2H_2)^{2+}$ ($n = 1$ or 2) as a function of $E$ were obtained from the breakdown curves multiplied by the measured total parent ion $C_{14}H_{10}^{2+}$ population distribution (the sum given in Fig. 5). For the comparison between the experiment and the simulation, we considered that the so-called $(C_{14}H_{10}-2H)^{2+}$ experimental distribution included the minor contribution of $(C_{14}H_{10}-H)^{2+}$.

FIG. 6. Simplified dissociation scheme of $C_{14}H_{10}^{2+}$ including the main dissociation channels only.

FIG. 7. (Color online) Calculated breakdown curves: Relative probability versus the excitation energy for $C_{14}H_{10}^{2+}$ parent ions to decay to $(C_{14}H_{10}-H)^{2+}$, $(C_{14}H_{10}-2H)^{2+}$, $(C_{14}H_{10}-C_2H_2)^{2+}$, and $(C_{14}H_{10}-2C_2H_2)^{2+}$ for a time windows $t_{\text{stat}} = 1 \mu$s.
which was actually embedded in the low-energy end of the measured $(\text{C}_{14}\text{H}_{10}-2\text{H})^{2+}$ curve. The simulated distributions were fitted to the experimental data (Fig. 8) by adjusting $E_d$ and $A_d$ for all involved channels. The obtained $E_d$ and $A_d$ parameters are presented in Table II. Although the number of adjustable parameters was large in the simulation, these parameters were not correlated and the solution was relatively unique. The good agreement between the simulation and the experimental distributions shows that the Arrhenius formula gives a quite satisfactory approximation for the energy dependence of the decay rates. This confirms the statistical nature of the involved dissociation processes.

In Table II, our estimated dissociation energies are compared with theoretical values published by Holm et al. [9]. The estimated $E_d$ values for $\text{C}_2\text{H}_2$ emission (2.5 eV) and for H abiotic (4.0 eV) from $(\text{C}_{14}\text{H}_{10})^{2+}$ are both lower than the adiabatic values $E_d$ (4.4 and 5 eV) of Ref. [9]. Comparing the two competitive dissociation processes ($\alpha'$ and $\alpha$) of the parent $(\text{C}_{14}\text{H}_{10})^{2+}$ corresponding respectively to the loss of H and the loss of $\text{C}_2\text{H}_2$, we note remarkable differences in the two sets of ($E_d$, $A_d$) values. The dissociation energy $E_d$ ($\alpha$) (2.5 eV) is lower than $E_d$ ($\alpha'$) (4.0 eV) and the preexponential factor $A_d$ ($\alpha'$) ($2 \times 10^{14}$ s$^{-1}$) is four orders of magnitude smaller than $A_d$ ($\alpha$) ($3 \times 10^{18}$ s$^{-1}$). These parameters led to simulated rate constants $k_\alpha$ ($E$) and $k_{\alpha'}$ ($E$) plotted in Fig. 9 as a function of the excitation energy $E$. The two-rate-energy dependence curves present a crossing point around 13 eV at $k = 5 \times 10^8$ s$^{-1}$. This feature is essential to reproduce the measured experimental population distributions that shift from the $\text{C}_2\text{H}_2$ loss channel at lower energy to the 2H loss channel at higher energy (Fig. 8). Due to the lower dissociation energy $E_d$ ($\alpha$) = 2.5 eV compared to $E_d$ ($\alpha'$) = 4.0 eV, $k_\alpha$ is larger than $k_{\alpha'}$ for $E < 13$ eV and the $\text{C}_2\text{H}_2$ loss channel is dominant. Owing to the large preexponential factor $A_d$ ($\alpha'$), at higher energy ($E > 13$ eV), $k_{\alpha'}$ becomes larger than $k_\alpha$ and the H loss channel is expected to be dominant. The fact that the 2H loss turns out to be the dominant channel around 14 eV implies that the loss of a H from $(\text{C}_{14}\text{H}_{10}-\text{H})^{2+}$ is a low-energy-cost process that occurs easily. Indeed a low dissociation energy, $E_d$ ($\beta'$) = 2.4 eV, has been obtained in the simulation. Although the expected weak $(\text{C}_{14}\text{H}_{10}-\text{H})^{2+}$ peak (Fig. 8) was not resolved in our spectrum, its presence in the modeling as an intermediate step was crucial to ensure the population of $(\text{C}_{14}\text{H}_{10}-2\text{H})^{2+}$ around the measured mean excitation energy 13.8 eV. Considering a part of the $(\text{C}_{14}\text{H}_{10}-2\text{H})^{2+}$ population as due to a one-step H2 loss process, this process would be in direct competition with the dominant $\text{C}_2\text{H}_2$ loss channel. Significant yield could be expected only if the dissociation rate of H2 loss is comparable to that of $\text{C}_2\text{H}_2$ loss. This should lead to a noteworthy shift of the $(\text{C}_{14}\text{H}_{10}-2\text{H})^{2+}$ population to a lower energy region around the mean energy (10 eV) for the $(\text{C}_{14}\text{H}_{10}-\text{C}_2\text{H}_2)^{2+}$ channel. Such an energy shift is not observed in our experiment [Figs. 4(c), 4(d)]. The direct H2 loss channel can be considered therefore as negligible. Similar reasoning holds for the direct C2H2 loss. This justifies the exclusion of the direct H2 or C2H2 loss channels in our modeling.

The rate-energy dependences $k(E)$ for H and C2H2 loss competing pathways have been estimated by Ling et al. [14] with a more sophisticated Rice–Ramsperger–Kassel–Marcus Quasi-Equilibrium Theory (RRKM-QET) statistical model for singly charged anthracene molecules using photoionization efficiency data. In this previous work, only the increasing part of the calculated breakdown curves could be fitted with the experimental data, and the comparison between the calculation and the experiment was based on the hypothesis that the energy carried by the incident photon was totally shared between the ionization potential ($I_1$) and the excitation energy ($E$) of the molecule, $hv = I_1 + E$. The kinetic energy of the ionized electron was neglected in the modeling. In contrast, the present work provides a complete fitting of the experimental data by the calculated breakdown curve profiles. This allows us to precisely follow the branching ratios of two competing molecular dissociation channels versus the excitation energy and to get more reliable rate-energy dependence $k(E)$ curves. In the internal excitation energy range from 10 to 20 eV, our

![FIG. 8. (Color online) Comparison between experimental (solid lines) and simulated (dashed lines) distributions for $(\text{C}_{14}\text{H}_{10})^{2+}$, $(\text{C}_{14}\text{H}_{10}-\text{H})^{2+}$, $(\text{C}_{14}\text{H}_{10}-2\text{H})^{2+}$, $(\text{C}_{14}\text{H}_{10}-\text{C}_2\text{H}_2)^{2+}$, and $(\text{C}_{14}\text{H}_{10}-2\text{C}_2\text{H}_2)^{2+}$. Experimental distribution of $(\text{C}_{14}\text{H}_{10}-2\text{H})^{2+}$ includes the small contribution of $(\text{C}_{14}\text{H}_{10}-\text{H})^{2+}$.](image)

**TABLE II.** Experimental and theoretical dissociation energies $E_d$ and preexponential factors $A_d$ for different fragmentation channels.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Lost fragment</th>
<th>$E_d$ (eV)</th>
<th>$A_d$ (s$^{-1}$)</th>
<th>$E_{\text{adiabatic}}$ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>$-\text{C}_2\text{H}_2$</td>
<td>2.5</td>
<td>$2 \times 10^{14}$</td>
<td>4.4</td>
</tr>
<tr>
<td>$\alpha'$</td>
<td>$-\text{H}$</td>
<td>4.0</td>
<td>$3 \times 10^{18}$</td>
<td>5</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$-2(\text{C}_2\text{H}_2)$</td>
<td>2.8</td>
<td>$2 \times 10^{14}$</td>
<td>4</td>
</tr>
<tr>
<td>$\beta'$</td>
<td>$-2\text{H}$</td>
<td>2.4</td>
<td>$2 \times 10^{14}$</td>
<td>4</td>
</tr>
</tbody>
</table>

$^a$Reference [9].
rate constants $k(E)$ are of comparable order of magnitude as those found for $C_{14}H_{10}^{+}$ dissociation in Ref. [14]. However in the case of $C_{14}H_{10}^{+}$, minor differences in rate-energy dependence curves have been observed for the competing H and C$_2$H$_2$ loss channels. The authors have concluded that both reactions were characterized by loose transition states. In the case of doubly charged anthracene, the crossing point (Fig. 9) of the rate-energy dependence curves is characteristic for two types of competing pathways in unimolecular ion decomposition. The H loss pathway, a fast process with high preexponential factor and large activation energy, can be attributed to a direct kinetically favored fragmentation process involving loose transition states. In contrast, the C$_2$H$_2$ loss corresponds typically to a thermodynamically favored reaction following rearrangement involving tight transition states.

V. CONCLUSION

We have studied the fragmentation of doubly charged $C_{14}H_{10}^{2+}$ molecular ions produced by DCT in collisions with fluorine projectiles using the CIDE method. The mean excitation energy of the parent $C_{14}H_{10}^{2+}$ ions related to different dissociation channels has been measured. We have demonstrated that in a time window of about 1 $\mu$s, $C_{14}H_{10}^{2+}$ is still stable with mean excitation energy as high as 8.5 eV. This value is much larger than the dissociation energy $E_d$ of the lowest-energy-cost C$_2$H$_2$ loss channel showing the high stability of the anthracene molecule versus dissociation. We have found that a small fraction of $C_{14}H_{10}^{2+}$ molecules (16%) decays by asymmetrical fission processes and the major part dissociates by the loss of neutrals. In contrary to $C_{14}H_{10}^{+}$ fragmentation in which the rate constants are very close for the competing H and C$_2$H$_2$ loss channels, for $C_{14}H_{10}^{2+}$, the emission of C$_2$H$_2$ is dominant at low excitation energy (<13 eV) and the emission of H followed by the loss of another H prevails at higher energy (>13 eV). The measured population distributions of the predominant neutral evaporation via mH or nC$_2$H$_2$ ($n = 1, 2$) emission from $C_{14}H_{10}^{2+}$ have been fitted by a statistical dissociation model for the dissociation rates and cascade model. The simulated rate-energy dependences of the two primary competing pathways, i.e., the loss of H or C$_2$H$_2$ from $C_{14}H_{10}^{2+}$, present a crossing point for $E = 13$ eV ($k = 5 \times 10^9$ s$^{-1}$). This feature is characteristic of two different fragmentation types: a direct cleavage for the loss of H and a rearrangement process for the loss of C$_2$H$_2$.

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Length effects in VUV photofragmentation of protonated peptides

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We have studied photoionization of protonated synthetic peptides YGnF (n = 0, 1, 3, 5, 10). Photon energies ranging from 8 to 30 eV were used. For YGnF peptides up to n = 5 small fragment ions related to the sidechains of the aromatic terminal amino acids Y and F dominate the fragmentation patterns. The associated yields scale with total photoabsorption cross section, demonstrating efficient hole migration towards the terminal amino acids upon photoionization of the peptide backbone. For n = 10 the side-chain loss channel is quenched and a series of large dications appear.

Vacuum ultraviolet (VUV) and extreme ultraviolet (XUV) photophysics of isolated biomolecules are of great interest for astrobiology and radiobiology. Key issues are e.g. ion chemistry in the interstellar medium, transport of free biomolecules from space to earth and their possible role in the development of life, molecular mechanisms of biological radiation damage and fundamental photophysics of complex molecules. In this context, charge migration is of particular interest.

Intramolecular charge transfer processes can involve conformational changes and accordingly occur at relatively long timescales.1 However, in groundbreaking experiments Weinkauf et al.2,3 have identified photoactivated fast hole migration in free peptide radical cations which is facilitated by the close energetic spacing of electronic states and their efficient coupling. Theoretical studies predict ultrafast (few fs or even sub-fs) hole migration processes over peptide bonds driven by electron correlation4,5 but direct experimental verification is still lacking. A promising route to investigate charge migration in biomolecules in a time-resolved fashion is XUV or VUV single-photon ionization. Very recently, Jiang et al. investigated ionization of gas-phase acetylene using 30 fs pulses of 38 eV photons from the free electron laser FLASH. Fast non-radiative decay via isomerization was observed.6 Ideally, a similar technique should be applied to peptides but dense gas-phase targets of these fragile non-volatile molecules are difficult to produce. This problem can be overcome by trapping techniques.

Over the past 10 years visible and UV laser spectroscopy of the protonated and deprotonated trapped peptide and protein ions has received considerable attention (for a review, see ref. 7).

First VUV photoionization and photofragmentation studies on these systems using synchrotron radiation have only been reported very recently.8–10 For the protonated peptide leucine enkephalin (amino acid sequence tyrosine-glycine-glycine-phenylalanine-leucine, YGGFL) we found that VUV photoionization predominantly leads to formation of ions related to the aromatic tyrosine (Y) and phenylalanine (F) sidechains.

Here we investigate the formation of sidechain-related fragment ions in more detail with a focus on charge migration. Protonated synthetic peptides YGnF are used. Variation of the number of glycine (G) residues n allows for systematic investigation of the effect of peptide length on the formation of sidechain ions. Fragmentation of peptides can often be understood in terms of statistical models such as quasi-equilibrium theory of unimolecular reactions and accordingly breakup patterns are expected to vary strongly with peptide length.11 For large peptides where statistical fragmentation should be weak, non-statistical fragmentation processes have been proposed.12 Peptide-size could also affect charge migration, e.g. if it is Coulomb driven.

The experimental technique is described in detail elsewhere.8 Briefly, singly protonated synthetic peptides [YGnF + H]+ (n = 0, 1, 3, 5, 10, for 3 see Fig. 1) were generated in an electrospray ionization source (ESI). A radiofrequency (RF) ion funnel was used to collect the ions and transport them into a collisionally focusing RF-only quadrupole. The peptide ions were mass selected in a RF-quadrupole mass analyzer and transferred through an end-cap into a 3D RF quadrupole ion trap. A He buffer gas was applied during the trap loading period for collisional cooling of the trapped ions. Typically, the trap was loaded with protonated peptides for a couple of 100 ms, before the ESI beam and the He buffer gas flow were stopped. The He gas was then pumped down for about 200 ms.

VUV and XUV photons from the U125 undulator at the BESSY II synchrotron (Berlin, Germany) were energy selected using a normal-incidence monochromator. The photon beam crossed the RF-trap through the ring electrode with its typical 100 μm × 120 μm focus located in the trap center. The photon flux was monitored on a silicon p-n junction photodiode. A mechanical shutter was used to expose the trapped protonated peptides for 0.1–1 s to the photon beam until a maximum number of 10% of the trap content underwent photon absorption. A second He buffer gas pulse was used to cool off fragment kinetic energies before the trap content was extracted into a linear time-of-flight mass spectrometer (M/ΔM = 200). Small fragments (m < 80 u) were not trapped. For each mass scan

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**References**

(ESI on, photons on (i)), subsequent subtraction of a scan of the residual-gas photoionization products (ESI off, photons on (ii)) yields the net effect of peptide irradiation. The target density was determined by the C, O and N 2p atomic orbitals. 16 For gas-phase Y and F, molecular orbitals localized on the Y and F aromatic sidechains. 15 As obvious from Fig. 3, the overall cross sections continuously drop.

The Y sidechain (Yside, m = 107) becomes the second largest peak. Excep for [YG3F+H]+ for all peptides almost exclusively fragments from the aromatic amino acids Y and F are observed, i.e. masses smaller than 200 u. Fig. 2 illustrates this for hν = 20 eV. This is consistent with our earlier findings for VUV photoionization and keV ion induced ionization of the pentapeptide leucine enkephalin (YGGFL). 8, 13 For n = 10 doubly charged cations become relevant (see zoom in Fig. 2). For this peptide also larger fragments due to backbone scission of the peptide occur in sizeable amounts.

Vertical ionization potentials of protonated peptides containing Y, G and F are typically around 9 eV with the highest occupied molecular orbitals localized on the Y and F aromatic sidechains. Above hν = 9 eV vertical ionization of [YG3F+H]+ is thus possible but competes with non-ionizing photodissociation processes following e.g. valence transitions and transitions to Rydberg states. Since VUV photoabsorption thus leads to photoionization or photodissociation, loss of parent [YG3F+H]+ from the trap is directly connected to the total photoabsorption cross section σexp(n), if the initial trap content, photon flux and irradiation time are known. The σexp(n) are displayed in Fig. 3 as a function of photon energy with σexp(0) set to unity at hν = 17 eV.

For hν = 8–13 eV, σexp(n) is almost constant indicating that increasing cross sections for photoionization of outer valence orbitals and decreasing cross section for photodissociation approximately cancel. In this energy range photoionization can be due to the π1 and π2 orbitals of the two aromatic rings (the HOMOs in [YG3F+H]+). 14 From the central Gm part of the [YG3F+H]+, nonbonding orbitals and π orbitals in the peptide groups strongly contribute, as observed in photoemission studies on GG dipeptides. 15 Above ~13 eV, σexp(n) increases strongly with hν to reach a broad maximum for hν ≈ 16–18 eV. GG photoemission spectra exhibit two broad maxima for electron binding energies between 13–15.5 eV and 15.5–19 eV due to σ orbitals formed by the C, O and N 2p atomic orbitals. 16 For gas-phase Y and F, broad photoemission features between 13–20 eV are also due to orbitals arising from the atomic 2p orbitals. 14 For higher energies, atomic C (2s) valence orbitals become accessible but the overall cross sections continuously drop.

As obvious from Fig. 3, σexp(n) strongly increases with n. YGGF(C8H9, 2H20, 3eN2, O4+) photoabsorption cross sections can be estimated by adding atomic cross sections. 17 For hν = 16.7 eV (in the σexp(n) maximum region) this procedure yields the cross sections σatomic(n) given in Table 1. The addition of atomic photoabsorption cross sections neglects the molecular nature of the target peptides and e.g. underestimates experimental data for benzene by a factor of two in the valence region. 18 Alternatively, we have estimated the [YG3F+H]+ absolute...
Fig. 3  Shaded area: \( \sigma_{\text{tot}}^{\text{atom}}(n) \) for [YG\(_{10}\)F + H\(^+\)]\(^+\) as a function of photon energy for \( n = 0, 1, 3, 5, 10 \). The bars indicate \( \sigma_{\text{tot}}^{\text{amino}}(n) \) at 16.7 eV and scaled to the experimental data for \( n = 0 \). Circles: cross sections \( \sigma_{\text{Y,F}}^{\text{atom}}(n) \) for production of Y, F related fragment ions. Squares (\( n = 10 \)): sum of \( \sigma_{\text{Y,F}}^{\text{atom}}(n) \) and the cross section for production of doubly charged fragments \( \sigma_{\text{dication}} \).

Photodissociation cross sections by adding the available experimental data for the constituent amino acids G and F\(^{19} \) and assumed \( \sigma_{Y} \approx \sigma_{F} \). At the same photon energy of 16.7 eV the values for \( \sigma_{\text{tot}}^{\text{amino}}(n) = \sigma_{Y} + n \sigma_{G} \) are given in Table 1. \( \sigma_{\text{tot}}^{\text{amino}}(n) \) probably overestimates peptide cross sections slightly as each peptide bond implies the loss of an H\(_2\)O unit. The main issue here is to compare the \( n \)-dependence of \( \sigma_{\text{tot}}^{\text{atom}}(n) \) and \( \sigma_{\text{tot}}^{\text{amino}}(n) \) with the experimental data. Scaled to the data for \( n = 0 \), the \( \sigma_{\text{tot}}^{\text{atom}}(n) \) and the \( \sigma_{\text{tot}}^{\text{amino}}(n) \) are almost equal (see Table 1). Scaled to the experimental data for \( n = 0 \), the \( \sigma_{\text{tot}}^{\text{amino}}(n) \) are added as shaded bars in Fig. 3.

The \( \sigma_{\text{tot}}^{\text{atom}}(n) \) thus scales with peptide cation length \( n \) indicating that the aromatic sidechains do not seem to play a special role. This is fundamentally different from the UV range, where aromatic sidechains such as those of Y and F\(^{20} \) can exceed \( \sigma_{\text{Y,F}}^{\text{atom}}(n) \). For \( n = 10 \) (see Fig. 3 and Table 1) \( \sigma_{\text{Y,F}}^{\text{atom}}(n) \) dropped back to values comparable to the \( n = 0 \) case. This decrease is remarkable as it indicates that not even holes created on the G units directly adjacent to the Y and F termini are subject to fast migration. Particularly for \( h\nu = 14-16 \) eV the yield of doubly charged cations \( \sigma_{\text{dication}} \) defined as the sum of the peak integrals \([YG_{10}\text{F} + H ]^+ + [YG_{10}\text{F} + H -10^{-7}]^+\), \([b12-107]^+\) and \(b11^+\), see Fig. 2) is exceeding \( \sigma_{\text{Y,F}}^{\text{atom}}(n) \). Very recently Milosavljević et al.\(^{21} \) reported that VUV photoionization of multiply protonated large protein cytochrome c (\( m \approx 12000 \) u) equally leads to formation of intact parent cations or to loss of a CO\(_2\) unit. Apparently, for YG\(_{10}\)F and \( n = 10 \) we witness the transition to the large peptide regime. Holes induced by photoionization of the G sites are not subject to fast migration towards the aromatic terminal amino acids anymore.

Based on the available data it is impossible to identify the exact mechanism of hole migration and its quenching for \( n = 10 \). Slagl et al.\(^{12} \) for instance discuss the possibility of charge transport along the peptide due to rapid (\( \sim 100 \) fs) and almost barrierless dihedral rotation occurring before IVR. In this model, it is conceivable that a G\(_{10}\) chain is simply too long to energetically allow for charge transport. On the other hand, electron correlation driven fast charge migration for instance can proceed via very different mechanisms\(^{22,23} \) and conformational differences cause variations in timescale ranging from the few fs regime to more than 100 fs.\(^{24} \) Last but not least, charge migration.

### Table 1

Photoabsorption cross sections at \( h\nu = 16.7 \) eV (in \( 10^{-16} \) cm\(^2\)) from addition of atomic cross sections\(^{13} \) \( \sigma_{\text{atom}}^{\text{ion}}(n) \) and addition of amino acid cross sections\(^{19} \) \( \sigma_{\text{amino}}^{\text{ion}}(n) \). The remaining columns display cross section ratios between YG\(_{10}\)F and YF:

<table>
<thead>
<tr>
<th>( n )</th>
<th>( \sigma_{\text{atom}}^{\text{ion}}(n) )</th>
<th>( \sigma_{\text{amino}}^{\text{ion}}(n) )</th>
<th>( \sigma_{\text{tot}}^{\text{ion}}(n)/\sigma_{\text{tot}}^{\text{ion}}(1) )</th>
<th>( \sigma_{\text{tot}}^{\text{amino}}(n)/\sigma_{\text{tot}}^{\text{amino}}(1) )</th>
<th>( \sigma_{\text{Y,F}}^{\text{atom}}(n) )</th>
<th>( \sigma_{\text{Y,F}}^{\text{amino}}(n) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.9</td>
<td>5.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>3.4</td>
<td>5.8</td>
<td>1.17</td>
<td>1.15</td>
<td>1.11</td>
<td>1.31</td>
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</tr>
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<td>12.6</td>
<td>2.71</td>
<td>2.5</td>
<td>2.32</td>
<td>0.78</td>
</tr>
</tbody>
</table>
could also be driven by the Coulomb repulsion away from the existing protonation site and also here, size and conformation are expected to play key roles. Future studies on peptide photoionization must therefore aim for identification of the migration mechanism. Ideally coincident measurement of fragment ion masses and photoelectron kinetic energies to determine the exact excitation energy transferred to the molecule has to be combined with a pump–probe scheme. The latter is feasible at free electron lasers and would give access to the time evolution of charge migration and fragmentation.

To summarize, we investigated VUV photoionization of [YG₉F + H]⁺ peptide cations for n = 0, 1, 3, 5 and 10 for photon energies hν = 8–30 eV. σ_{exc}(n) was found to increase systematically with n as expected from the addition of cross sections of the constituent amino acids. Up to n = 5 the fragmentation pattern is dominated by formation of cations related to the aromatic sidechains of the terminal Y and F residues, i.e. photoabsorption on the G residues is followed by charge migration to the terminal amino acids. For n = 10 this charge migration process was found to be quenched. Instead, large dications and large singly charged fragments were observed while yields of sidechain related fragments get reduced.

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References
Photodissociation of protonated leucine-enkephalin in the VUV range of 8–40 eV

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Until now, photodissociation studies on free complex protonated peptides were limited to the UV wavelength range accessible by intense lasers. We have studied photodissociation of gas-phase protonated leucine–enkephalin cations for vacuum ultraviolet (VUV) photons energies ranging from 8 to 40 eV. We report time-of-flight mass spectra of the photofragments and various photofragment yields as a function of photon energy. For sub-ionization energies our results are in line with existing studies on UV photodissociation of leucine–enkephalin. For photon energies exceeding 10 eV we could identify a new dissociation scheme in which photoabsorption leads to a fast loss of the tyrosine side chain. This loss process leads to the formation of a residual peptide that is remarkably cold internally. © 2011 American Institute of Physics. [doi:10.1063/1.3515301]

I. INTRODUCTION

Amino acids are the building blocks of peptides and proteins present in all living organisms on Earth. Since amino acids were found in some meteorites1–3 and are likely to exist in the interstellar medium (ISM),4,5 transport of prebiotic and biotic molecules from outer space to Earth is considered an alternative to the conventional assumption of purely Earth bound development of life.

Many studies on photo-induced ionization and fragmentation of amino acids have been conducted,6–8 for instance to be able to recognize signatures of these molecules in the ISM, in comets, and in planetary atmospheres or to understand the mechanisms behind the in–space formation of amino acids. It was already shown that amino acid synthesis from simpler organic molecules can be induced by interaction with cosmic rays,9,10 spark discharges,11 ultraviolet (UV)/vacuum ultraviolet (VUV) radiation or simply by thermal excitation.12,13 Bernstein et al.14 and Caro et al.15 synthesized several amino acids by UV irradiation of interstellar ice models and Nuevo et al.16 even concluded that amino acids are always formed when interstellar ice models containing C, H, O, and N are irradiated with VUV photons. Even peptide bond formation was observed under VUV irradiation of dry amino acid films or amino acids in icy matrices,17,18 implying that peptides could be formed e.g., on interstellar dust grains or small solar system bodies. An obvious next step now is to investigate the photo stability of peptides. Studies during long duration space missions indicated that dipeptides and the tripeptide tri-L-leucine thioethylester have higher stability upon UV-irradiation than amino acids.19,20 But how photo stable were the early peptides on Earth and is it possible that intact early peptides have been transported from space to Earth in the gas phase? Van der Gulik et al.21 assume early functional prebiotic peptides to be 3–8 amino acids long. We have therefore chosen the pentapeptide leucine–enkephalin (leu–enk) as an ideal subject for studying peptide response upon photoabsorption. Since photoabsorption cross-section of amino acids typically peak in the VUV range, i.e., at wavelengths shorter than 200 nm,22 in this study we will focus on VUV-photon absorption. VUV wavelengths are particularly interesting also because in this wavelength range the luminosity of the early Sun was 2 orders of magnitude higher than today so that VUV/UV is assumed to be the most intense radiation on the early Earth during the Hadean period (4.6–3.8 gigayears ago) with no or very dilute atmosphere present.23

In the following, we present the first systematic VUV photodissociation study of a free protonated peptide. Leu–enk has the additional advantage that it has already been investigated by a whole arsenal of mass-spectrometric tools. The obtained fragmentation pattern and yields will be interpreted in the context of existing data on leu–enk surface-induced dissociation (SID),24 blackbody infrared radiative dissociation (BIRD),25 laser-induced dissociation (LID),26 multiphoton-induced27 and collision-induced (CID)28 mass spectra. The influence of the electronic structure of the peptide and its constituent amino acids on the dissociation and ionization will be discussed.

II. EXPERIMENT

Recently, we have developed a new apparatus (for a sketch, see Fig. 1) in which a home-built electrospray ionization (ESI) source is combined with a radiofrequency (RF) trap and a time-of-flight (TOF) mass spectrometer. This setup has been interfaced with a VUV photon beamline of the third generation synchrotron facility BESSY II in Berlin. Tunable
VUV photons in the energy range of 8–40 eV were obtained from the quasi periodic undulator U125/2 (Ref. 29), which consists of 32 dipole magnet periods each 125 mm long, in combination with the 10 m focal length normal incidence monochromator (NIM). In order to achieve maximum photon flux a relatively low resolution of 300 lines/mm grating was employed.

A brief description of the experimental setup follows, which will be described in more detail in a later publication. The singly protonated cations of the pentapeptide (leu–enk, amino acid sequence: YGGFL, m = 555.62 amu, a scheme is shown in Fig. 2) were generated in the ESI source from a ∼30 μM methanol solution with 1% formic acid. The ions were then passed through a collisionally focusing RF-only quadrupole. After phase space compression, the peptide ions passed an RF-quadrupole mass analyzer. Mass selected protonated leu–enk ions entered a quadrupole ion trap through its endcap (see Fig. 1). The trapped peptides served as a target for the VUV photons.

The base pressure inside the trap chamber was 1 × 10⁻⁹ mbar. For collisional cooling of the peptide ions, a He-buffer gas pulse was applied during the trap loading period. The estimated pressure inside the trap increased to 1 × 10⁻³ mbar, and the trap was typically loaded with protonated peptides over a period of 400 ms. The ion beam was then blocked by means of a 100 V skimmer bias. At the same time the solenoid valve, controlling the He-buffer gas flow, was closed and the pressure in the trap decreased to about 1 × 10⁻⁶ mbar.

The VUV photons intersected the Paul trap through the ring electrode. The photon beam focus was chosen to lie in the center of the RF-trap. The geometric beam cross section was 100 μm × 120 μm (at 25 eV with 20 μm slit width). The photon flux was monitored using a GaAsP–Schottky diode located 23 cm behind the trap center. The protonated peptides were then exposed for about 0.1–1 s to the photon beam which was chopped by a mechanical shutter with a 14 mm aperture. To avoid sizeable contributions of multiple absorption processes, the conditions were chosen such that a total of about 10% of the trapped protonated peptides were dissociated by interactions with photons, i.e., less than approximately 10% of the dissociated peptides underwent absorption of more than one photon. After 80 ms of irradiation the trapped protonated peptides and their cationic dissociation products were extracted into a linear TOF mass spectrometer (M/ΔM = 200) by applying a bias voltage (U bias = ± 200 V, duration: 5 μs) to the RF-trap endcaps. The ions were detected by a microchannel-plate detector with the front plate biased to −2 kV and the anode kept at ground potential. The detector signal was recorded by a 1 GHz digitizer.

Despite the low background pressure and a liquid nitrogen cooled cryo-trap close to the RF-trap, contamination of the buffer gas or neutral molecules from the ESI source may have contributed to the mass spectra. To extract the mass spectrum due to leu–enk fragmentation only, the data acquisition was divided into successive cycles of three mass scans. In each cycle, first the TOF spectrum resulting from VUV photon irradiation of the trapped protonated peptides and neutral residual gas was recorded (inclusive scan). To obtain the net effect of photon irradiation upon the trapped protonated peptides, in a second scan the photon beam was blocked and a TOF spectrum of the initial trap content only was recorded. For the third scan, the ESI source was switched off and the TOF spectrum resulting from the photoionization of residual gas molecules was recorded. The latter two spectra were then subtracted from the inclusive scan. A three-scan cycle took about 3 up to 6 s. To obtain the final mass spectra a series of 2000–6000 cycles was accumulated for each photon energy.

In order to be able to directly compare peak intensities and integrals from mass spectra obtained at different photon energies, it was necessary to normalize the mass spectra. The relative photon flux was determined from the photocurrent of the GaAs photodiode, divided by its photonenergy dependent quantum efficiency. Note, that the photodiode had not recently been calibrated and accordingly no very reliable numbers for the absolute flux could be obtained. The density of the leu–enk cation target was determined from the integral of the unirradiated leu–enk peak in the TOF spectrum. Trap depth and RF-frequency were not changed during the experimental campaign, i.e., the target volume was constant. Typically ion-clouds trapped in RF-traps at the parameters used in our study were about 1 mm in diameter. The exact target volume

![FIG. 1. Experimental setup.](image1)

![FIG. 2. Structure of leucine–enkephalin with its five constituent amino acids and their three-letter code (bold) and one-letter code indicated. The nomenclature of the fragments as well as the immonium ions are displayed.](image2)
is difficult to determine. However, with relative photon flux, relative target density and irradiation time known, the spectra could be normalized and peak integrals from the mass spectra could be interpreted as relative photodissociation cross-sections.

III. RESULTS

Typical normalized fragment cation mass spectra obtained after irradiation of leu–enk with 8, 15, and 20 eV photons are shown in Fig. 3. Note that the intensity range is about a factor of 15 smaller for the 8 eV spectrum as compared to the 15 and 20 eV spectra. Particularly the spectra at 15 and 20 eV exhibit some resemblance to our recent keV ion-induced dissociation (KID) results.

In general in the photon energy range 10–40 eV, the spectra are dominated by fragments with m/q = 80–240 amu. The immonium ions at 86 (L), 120 (F), 136 (Y), and the common fragments of these groups (91 and 107) are strongly contributing. For the photon energy range 10–40 eV the VUV induced fragmentation spectra differ strongly from what is observed in CID (Ref. 28), SID (Ref. 24) and also IR multiphoton absorption where fragments with m/q exceeding 350 dominate the spectra and immonium fragments are usually weak.

In the following paragraphs the spectra obtained at the photon energies (hv) 8, 15, and 20 eV will be described in more detail.

A. 8 eV: Below ionization threshold

If the photon energy falls short of the ionization energy (IE), photoabsorption transfers the protonated peptide into an electronically excited state. We have used density functional theory (DFT) calculations (B3LYP level, 6-31+G(d,p) basis set) using GAUSSIAN 03 (Ref. 33) to determine an IE = 8.87 eV for the lowest energy conformer of protonated leu–enk determined by IR spectroscopy and quantum chemical calculations.34

The fragmentation patterns of leu–enk obtained with 8 and 9 eV [Fig. 3(a)] photons are very different from the spectra for all the other photon energies, most probably because at these low photon energies the leu–enk cation cannot be directly photoionized. The peak at m/q = 278/279 assigned to b7/y2 (possibly with a small contribution of the (M+H)2+ radical dication) is the strongest peak followed by the immonium ion with m/q = 120 (F). The fragments a4 = 397 and b4 = 425 which are most abundant when dissociation techniques such as CID and BIRD (Ref. 25) are used are either weak (a4) or absent (b4).

The peaks with mass-to-charge ratios of 380 (a5–NH3), 323 (380-glycine residue), 233 (380-phenylalanine residue), 217 (380-tyrosine residue), 262 (GGF), 205 (GF), and 177 (GF–CO) are due to internal fragments. Probably these fragments result from a cyclic rearrangement of the a5 intermediate as identified by Vachet et al.35 for CID of leu–enk investigated by multiple stage mass analysis in a quadrupole ion trap. At higher photon energies, all these fragments with the exception of GF are suppressed.

The peaks at m/q = 449 and 465, corresponding to tyrosine side chain loss (107) and phenylalanine side chain loss (91) respectively, are clearly visible for 8 eV photon energy. These peaks are also observed by Tabarin and co-workers,26 who employed LID at photon energies between 4.4 eV (280 nm) and 5.6 eV (220 nm) but are usually not present in CID spectra.

![FIG. 3. VUV photon-induced mass spectra of protonated leu–enk at 8, 15, and 20 eV photon energy.](image-url)
 Fragment yields

An overview of the normalized leu–enk photodissociation TOF-spectra covering the whole range of photon energies between 8 and 40 eV is displayed as a color-coded contour plot in Fig. 4. The photon energies at which spectra were acquired are given on the y axis. The spectrum on top is a cut through the contour plot indicated by the white dotted line at 15 eV [identical with Fig. 3(b)]. The data in between measured photon energies have been obtained by mere interpolation. Note the nonlinear photon energy scale! It is obvious from Fig. 4 that all fragment peaks exhibit a strong photon-energy dependence. As mentioned before, at \( h\nu = 8 \) eV the relative fragment yields are weak and fragments reach their peak intensities between 10 and 30 eV. For photon energies exceeding 30 eV, the relative yields are decreasing again. Different fragments apparently peak at different photon energies. For instance, \( m/q = 120 \) (F) peaks at 15 eV whereas \( m/q = 107 \) (tyrosine side chain) peaks at 20–25 eV.

For a number of stronger fragments, photofragment yield curves are displayed in Fig. 5. The statistical uncertainties, originating from the statistics and data handling, are very small and mostly not even visible in the plots. Further systematic error sources, such as deviations from the calibration of the photodiode and contamination from higher harmonics from the undulator during irradiation at lower energies are not taken into account. All yields exhibit a broad peak which in most cases has a full width at half maximum of about 10 eV. Only for \( m/q = 120 \) (F) there is an absolute maximum at \( h\nu = 15 \) eV whereas the maximum is around \( h\nu = 20 \) eV for the remaining fragments. In addition, there might be a local maximum at \( h\nu = 15 \) eV photon energy for many fragment ions. The tyrosine side chain fragment \( (m/q = 107) \), the F-immonium ion and the internal fragment GF reach highest yields amongst the fragments.

Rather than by summation of all photofragment ion yields, the total photodissociation yield can be obtained by subtraction of the parent \((M+H)^+\) peak after irradiation from the parent peak prior to the irradiation. Figure 6 shows the normalized total photodissociation yield as a function of the photon energy. Again, the yield curve features a broad peak with a local maximum at about 15 eV and a total maximum at about 20 eV. Note, that due to a \( m/q \) dependence of the detection efficiency and due to multiplicity into two or more fragments, the total photodissociation yield is smaller than the sum of the fragment yields.

IV. DISCUSSION

In the photon energy range under study here \((8–40 \) eV), two different regimes of photoabsorption triggered dissociation processes have to be considered.

(I) For \( h\nu < \text{IE} \) vertical ionization is ruled out and mainly excitation processes contribute. (Note, that adiabatic channels such as ion-pair formation might also lead to photoion production below the vertical ionization threshold.) The dominating processes here are valence transitions. (To a smaller extent, resonant excitation into Rydberg orbitals can take place.) Three groups of chromophores contribute to valence transitions in peptides, namely the peptide bond itself, the aromatic side chains, and the terminal amine and carboxyl groups. However, it is the peptide bond that is expected to contribute dominantly in the 8–9 eV photon-energy range studied here.\(^{36}\) The peptide bond can be viewed as a four level system consisting of two doubly occupied \( \pi \)-orbitals \((\pi_1 \text{ and } \pi_2)\), the O lone pair and an antibonding empty \( \pi^* \) orbital with the \( \pi_1-\pi^* \) transition being responsible for the absorption around \( h\nu = 9 \) eV. At \( 8 \) eV, so-called charge transfer
transitions of a $\pi_2$ electron from one peptide bond into the $\pi^*$ orbital of an adjacent peptide bond can still contribute.\(^{37}\)

The initial photoexcitation processes can be followed by radiative de-excitation, intramolecular vibrational redistribution (IVR), direct dissociation through dissociative electronic states, and other processes, which can differ greatly in timescales involved. When we assume that the excitation of one of the peptide bonds is followed by IVR, we can directly compare our results to the SID studies by Laskin.\(^{24}\) For 50 eV protonated leu–enk collisions with a self-assembled alkane thiolate monolayer, a maximum of about 17% of the collision energy is transferred into the peptide\(^{38}\) implying about 8.5 eV of internal energy—about the same as deposited by 8 or 9 eV photoexcitation here. The SID spectrum, qualitatively similar to our photoexcitation data [see Fig. 3(a)], is dominated by 120 (F), 136 (Y), $b_3/y_2$, $a_4$, and $a_4$-NH\(_3\). Internal fragments GF-28, GF, GGF, and FYG are observed as well. Laskin\(^{24}\) found a strong time dependence of $a_4$-NH\(_3\) and FYG and concludes that these fragments are associated with the cyclic rearrangements previously mentioned. This cyclic $a_4$ rearrangement has already been invoked earlier by Vachet and co-workers.\(^{35}\) The fragments $b_3$, GF, and 120 (F) show no time dependence and are formed following entropically favored pathways.\(^{24}\)

Despite the good agreement in fragments observed, the fragment yields are very different: For SID, $a_4 = 397$ and $b_3 = 425$ are the strongest fragments whereas (F) and (Y) are weak. The opposite is true for our 8 eV photon data [Fig. 3(a)]. The reason for the difference could lie in the possibility of dissociation through repulsive electronic states occurring before IVR: At $h\nu = 8$ eV, we observe the relatively strongest contribution of $m/q = 449$ and $m/q = 465$ due to loss of the neutral Y-side chain and the neutral F-side chain, respectively. Tabarin et al.\(^{26}\) observed identical loss processes for photon energies between 4.4 and 5.6 eV after photodissociation in either of the tyrosine and phenylalanine chromophores. This was already earlier interpreted as an indication of fast dissociation occurring before internal vibrational redistribution (IVR).\(^{39}\) For our data this implies that sizeable absorption in the tyrosine and phenylalanine chromophores still takes place for photon energies of 8 or 9 eV.

(II) For $h\nu > IE$ the protonated peptide can be photoionized and a protonated leu–enk dication radical is formed. (The process in which a stronger bound electron can get photoexcited below the ionization threshold will not be considered

![FIG. 5. Protonated leu–enk photofragment ion yields as a function of photon energy for nine important fragments. All spectra have identical scales.](image1)

![FIG. 6. Total photodissociation yield of protonated leu–enk as a function of photon energy (defined as the photon-induced depletion of the parent ion signal).](image2)
because the subsequent dissociation dynamics probably resembles what was discussed for the (I) case and relatively small fragment yields are expected due to the higher excitation energies.)

Hence, when the photon energy passes the IE, a fundamentally different regime is reached. Instead of the hot singly charged system discussed in (I), an electron from a (local) highest occupied molecular orbital (HOMO) is photoionized and a comparably cold doubly charged system is formed. The close to threshold regime, has been explored, e.g., by Walter et al. In their experiments resonant two-photon absorption by neutral laser-desorbed smaller peptides only led to negligible fragmentation.

When the photon energy continues to increase, electrons from deeper lying molecular orbitals become accessible for ionization. This gives rise to the formation of an intermediate (leu-enk + H)\(^+\) complex in an electronically excited state. If IVR dominates, the excess energy \(E_{\text{ex}} \approx h\nu - \text{IE}\) remains in the (leu-enk + H)\(^+\) complex. How much excitation energy is needed to induce fragmentation of this intermediate system? Laskin showed that in protonated leu-enk typical fragmentation pathways leading, e.g., to GF formation open up for internal energies between 3 and 4 eV, if reaction times of the order of a second are employed. These reaction times are comparable to the ones used in the present study and our results can thus be explained within this framework:

For most fragments (except the \(m/q = 120\) (Y) and the \(m/q = 136\) (Y) immomun ions which will be discussed later) the yield is only increasing very weakly up to \(h\nu = 13\) eV from where it starts to increase sharply [see Fig. (5)]. Apparently here the internal energy overcomes the thresholds for dissociation following IVR. Most fragments display a local maximum at \(h\nu = 15\) eV as well as a broader and more intense maximum around \(h\nu = 20\) eV. It is known that the photoabsorption cross sections of larger peptides agree quite well with the absorption cross sections of the isolated amino acids, i.e., that photoabsorption remains a local process. It is thus instructive to compare our data with photofragment yield curves measured by Jochims et al. for neutral gas phase amino acids. Their photofragment curves e.g., for glycine exhibit inflections at each energy where a deeper lying molecular orbital becomes accessible (see also Ref. 42) and are qualitatively similar to our results. The photofragment yield curves thus depend on the energetic ordering of the valence molecular orbitals, i.e., the molecular density of states. It is useful to look into the photoelectron emission spectra from the four amino acids present in leu-enk to get a deeper insight into the origin of the fragment-yield curves presented in Fig. 5.

For glycine, the photoelectron emission spectrum obtained at 99 eV (Ref. 43) is dominated by intense peaks due to valence electrons at binding energies between 10 and 19 eV, with the lowest three being due to the N lone pair \(n_N\) (HOMO), the hydroxyl O lone pair \(n_O\) and the bonding carbonyl orbital \(\pi_{\text{CO}}\). The weaker peaks at higher binding energies are due to single electron ionizations. The same ordering is observed also for other aliphatic amino acids so we expect similar highest occupied molecular orbitals for leucine as well.

![FIG. 7. Comparison of the photoelectron data for the amino acids glycine (Ref. 43) (a), phenylalanine (Ref. 44) (b), and tyrosine (Ref. 43) (c) with the calculated molecular valence orbital density of leu-enk (d) and the fragment yields of GF (e), F (f), Y (g) and the 107 fragment of Y (h).](image-url)

The aromatic amino acids tyrosine and phenylalanine have a slightly different valence structure since the \(\pi\) orbitals on the phenyl (phenylalanine) and phenol (tyrosine) rings have lower ionization energies than the \(n_N\) orbitals. For phenylalanine, the phenyl \(\pi_1\) and \(\pi_2\) orbitals and the \(n_N\) lone pair coincide energetically at 9.5 eV whereas for tyrosine, the \(\pi_1\) peak is located at 8.5 eV whereas \(\pi_2\) and \(n_N\) are found at 9.6 eV. For both amino acids, the intense peaks originating from valence electrons span a binding energy range up to about 18 eV, i.e., the amino acid valence spectra qualitatively agree in the width of the peaks with the fragment yields measured here.

In the DFT calculations mentioned before, we have calculated the density of molecular valence orbitals for protonated leu-enk [see Fig. 7(d)]. The above mentioned photoelectron data for the different amino acids are displayed for comparison [see Figs. 7(a)–7(c)]. Clearly the peptide electronic structure overall resembles those of the amino acids. The three highest occupied molecular orbitals for protonated leu-enk are located on the phenyl ring (HOMO, HOMO-1) and on the phenol ring (HOMO-2). Even though the ordering deviates from the case of the isolated neutral amino acids, we find agreement in the sense that the aromatic side chains host the most weakly bound electrons. As mentioned above, in the context of dissociation following IVR, states with binding energies of about 13 eV and more are relevant, since only ionization from these states leads to dissociation. Figure 7(e) displays the GF yield (a typical fragment formed after IVR) for comparison with the protonated leu-enk density of states.
It is obvious that the rise of the fragment yield coincides with the second maximum of the density of states, where about 20 molecular orbitals are found between 13 and 15 eV. The further increase of the fragment yield up to photon energies of about 20 eV can be explained with the fact that up to this energy additional molecular orbitals become available for photoionization, i.e., the absorption cross section is expected to increase. Only a couple of states lie deeper than 20 eV and these are almost exclusively atomic 2s orbitals with small cross sections. Accordingly, the fragment yield decreases above 20 eV.

From Figs. 4, 7(f), and 7(g) it is obvious that above 9 eV (only) the \( m/q = 120 \) (F) and the \( m/q = 136 \) (Y) immonium ions are strongly increasing, i.e., these fragments are not formed following simple IVR. The yields of both immonium ions peak at \( h\nu \approx 15 \) eV which for (F) even is the absolute maximum in yield. The \( \pi \) orbitals from the aromatic side chains have low binding energies up to around 12 eV so that a large fraction of the ionization processes in this photon energy range will certainly originate from the aromatic groups. Probably in these cases fast dissociation via repulsive molecular states is more efficient than dissociation following IVR.

The most remarkable feature in particular well above the ionization threshold is the fact that \( m/q = 107 \) (the tyrosine side chain) shows up and becomes the strongest peak [Figs. 4 and 7(h)]. Note that to our knowledge for leu–enk this fragment ion has been only observed before in our previous KID studies. But this fragment ion also is the dominant peak in dissociative photoionization of neutral gas phase tyrosine.43,44 Loss of a charged aromatic side chain, i.e., breaking of the \( C_{\alpha} - C_{\beta} \) bond for a neutral tryptophan–gly\(_{\alpha}\) peptide has also been observed.45 Below the ionization threshold we infer loss of the neutral \( m/q = 107 \) tyrosine side chain (and of the \( m/q = 91 \) phenylalanine side chain) from the observed peak M-107 at \( m/q = 449 \) (and M-91 at \( m/q = 465 \)). Above threshold, the additional charge gives rise to a charge separation process. Also in this case, the process underlying the dissociation cannot be IVR but probably involves a repulsive molecular state.

Together with the 107 fragment ion, a number of N-terminal fragments are observed, which are due to backbone scission accompanied by the loss of the (N-terminal) tyrosine side chain, namely \((b_2 - 107)^+\), \((c_2 - 107)^+\), \((b_1 - 107)^+\), \((c_3 - 107)^+\), \((a_2 - 107)^+\), and \((b_2 - 107)^+\). In high energy CID studies, usually only side chain loss of the amino acid at which the backbone cleavage occurred is observed. Moreover, even in such cases loss or fragmentation of aromatic side chains is typically weak or even absent. In our KID study on protonated leu–enk on the other hand, we have observed similar dissociation dynamics.32

In case of photoionization the electron is removed from one of the HOMOs located on the tyr- and phe- side chains which apparently induces nonergodic fragmentation similar to the LID case, i.e., fast scission of the \( Y C_{\alpha} - C_{\beta} \) bond. Since during photoionization the electron is not excited but removed, the side chain is positively charged and appears as the dominant feature in the mass spectrum [Fig. 3(c)]. Note that Tabarin et al. accordingly did not observe this fragment ion in their LID spectra.26 The remaining protonated peptide cation then undergoes IVR before the excess excitation energy induces backbone scission according to the mobile proton model.47 Upon an increase of vibrational excitation energy, the proton attached to the remaining
peptide becomes mobile and samples various protonation sites within the molecule. This way, a fragmentation pattern as expected for CID but shifted to lower masses by 107 amu arises. Figure 8 visualizes the discussed sequence of processes.

This observation has interesting implications in the astrobiological context mentioned in the introduction. Figure 9 again displays the mass spectrum obtained for photoexcitation with 8 eV photons as well as the highlighted part of the 20 eV spectrum, that is due to \( m/q = 107 \) cation loss. For a better comparison, the latter spectrum has been shifted in \( m/q \) by 107. The most obvious finding is that for the 20 eV spectrum relatively more intense peaks are found at larger \( m/q \). In particular in the \( m/q = 107 \) cation loss case, the \( a_4 \) and \( b_4 \) fragments are very strong. The ratio of these peaks can be used to compare internal energies of the respective system before fragmentation occurred. Laskin\(^1\) showed for instance that in SID \( a_4 \) can be formed above 20 eV collision energy with a maximum at 45 eV. \( b_4 \) on the other hand can be formed above 10 eV and peaks at 35 eV. At photon energies of 8 or 9 eV (corresponding to 50 eV or more of collision energy in SID), we observe no \( b_4 \) fragment cations. For the case of \( m/q = 107 \) cation loss, equal \( a_4 \) and \( b_4 \) yields are observed, implying \( \sim 4.8 \) eV of internal energy.

The fast loss of the charged tyrosine side chain after 20 eV photoabsorption is thus an efficient mechanism to cool the remaining peptide. It is conceivable, that such loss processes facilitate survival of functional peptide substructures after absorption of very energetic photons.

V. CONCLUSION

We have shown that in the photon energy range 8–40 eV different regimes of dissociation processes, below and above IE, have to be considered. Below the ionization energy the photon energy (8–9 eV) is mainly absorbed by the peptide bonds which leads to slow fragmentation governed by IVR. Furthermore we explain differences in qualitatively similar SID spectra in this regime by the possibility of fast dissociation through repulsive states before IVR after absorption of the tyr- and phe- side chain chromophores.

Most fragment yields have a local maximum at a photon energy of 15 eV as well as an absolute broad maximum at 20 eV. This shape can be explained qualitatively with photoemission spectra of the constituent amino acids and the leu–enk density of molecular states.

At photon energies higher than the ionization energy the relative cross sections of most fragments show a substantial increase above \( h\nu = 13 \) eV where the internal energy of the intermediate ionized leu–enk overcomes the dissociation threshold following IVR. An exception are the immo- nium fragments F and Y where fast dissociation through repulsive states competes with IVR at lower energies.

The tyrosine side chain fragment at \( m/q = 107 \) shows up at ionization threshold and becomes the strongest peak. This fragment was the first time for leu–enk observed in our KID (Ref. 32) studies. Also here, the electron from the HOMO located on the tyrosine side chain is removed inducing fast fragmentation of the \( C_{\alpha}–C_{\beta} \) bond leading to a charged fragment.

With the loss of the tyrosine side chain fragment a number of N-terminal fragments are observed accompanied with the loss of the tyrosine side chain. From the ratio of the related \( a_4/b_4 \) fragments we could deduce the internal energy and concluded that the fast nonergodic 107 fragment ion loss efficiently cools the residual peptide.

This efficient cooling process seems to allow for survival of early functional peptide substructures under very energetic photon irradiation. Therefore, it is conceivable that substructures of peptides could survive on the early Earth and also transportation to it, providing a basis for the formation of new peptides.

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